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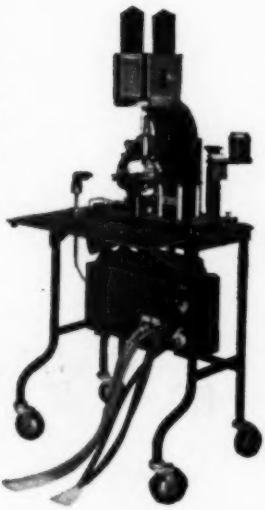

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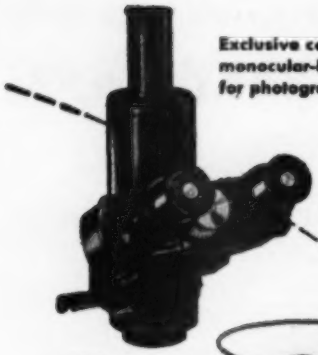

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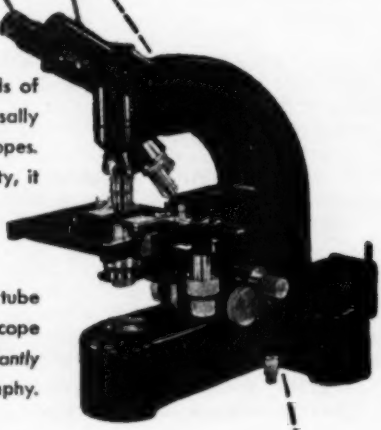

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LESIONS OF EPIPHYSEAL CARTILAGE AND SKELETAL MUSCLE IN RATS ON A DIET DEFICIENT IN POTASSIUM

JONATHAN COHEN, M.D.
ROBERT SCHWARTZ, M.D.
AND
WILLIAM M. WALLACE, M.D.
BOSTON

IN WEANLING rats the principal pathologic consequences of a dietary deficiency of potassium are lesions of the renal tubules and of the cardiac muscle. The purpose of this report is to describe lesions of skeletal muscle and epiphyseal cartilage which have not been noted previously. Smith, Black-Schaffer, and Lasater [1] have reported lesions of the skeletal muscle of dogs fed a potassium-deficient diet for more than 12 weeks, but an analogous diet did not produce similar lesions in rats. The lesions described resembled the well known entity of Zenker's degeneration of muscle and healed rapidly, with no scar formation, when potassium salts were added to the diet. Follis, Orent-Keiles, and McCollum,[2] in their authoritative study of the pathology of potassium deficiency in rats, found no lesions in the skeletal muscle or in the epiphyseal cartilage. They called attention to the variation in pathologic alterations produced by numerous diets, all deficient in potassium, and explained the discrepancies by deficiency of other nutriments in addition. The normally high content of potassium ions in skeletal muscle has focused much attention on the decrease of muscle potassium associated with potassium deficiency, and the absence of detectable pathologic changes has, therefore, been difficult to explain. Similarly, the retardation of growth of rats on potassium-deficient diets tends to draw attention to the centers of growth, the epiphyseal cartilages, for an explanation. While the diet used in the experiments to be described differed in some respects from those used in other pathological studies, it was thought to be adequate in all

Dr. Schwartz was Postdoctoral Fellow of the National Institutes of Health, United States Public Health Service, 1950-1951.

From the Division of Nutritional Research, Division of Laboratories and Research, and the Departments of Orthopedic Surgery and Pediatrics, The Children's Medical Center and Harvard Medical School.

This investigation was aided by grants from the Williams-Waterman Fund for the Combat of Dietary Disease, The Nutrition Foundation, Playtex Park Research Institute, and the National Heart Institute of the National Institutes of Health, United States Public Health Service.

1. Smith, S. G.; Black-Schaffer, B., and Lasater, T. E.: Potassium Deficiency Syndrome in the Rat and the Dog, Arch. Path. **49**:185, 1950.

2. Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V.: The Production of Cardiac and Renal Lesions in Rats by a Diet Extremely Deficient in Potassium, Am. J. Path. **18**:29, 1942.

necessary elements except potassium, and the detection of the muscle and cartilage lesions came somewhat as a surprise.

MATERIALS AND METHODS

Seventy-six weanling rats of the Hisaw strain, approximately evenly divided according to sex, were used in these experiments. They were housed in individual mesh cages and were acclimatized to their surroundings for a period varying from two to five days, during which their weight gain on a commercial diet [3] administered ad libitum was recorded. After this observation period, the potassium-deficient diet was administered to 60 of these animals for the duration of the experiment, while 8 animals received the potassium-deficient diet supplemented with potassium bicarbonate, and 8 control animals continued on the commercial diet. No paired feeding controls were run.

The general plan of the experiment was designed to follow the repair sequences after potassium deficiency had been induced, with particular emphasis on histological patterns in the heart and on tissue electrolyte changes. To that end a standard interval of dietary deficiency of 14 days for all rats in the experimental group was decided on. Six animals succumbed during this period, one at 7 days and the others at 11 to 13 days. Following this period, all the controls and 17 experimental animals were killed. The remaining rats were divided into four experimental repair groups, approximately equal in number. All of these received one day's therapy of injected potassium salt solution and were maintained on the deficient diet until killed. One group of seven rats was subjected to five intraperitoneal injections of potassium chloride at two-hourly intervals, the total dosage being equal to 17 mM./kg. These animals were killed at intervals of one to seven days thereafter. A second reparative group, consisting of 12 animals, was similarly treated with KCl in a dosage of 25 mM./kg., and a third group, consisting of 11 animals, was treated similarly with potassium acetate 25 mM./kg. The final group of 12 animals received KCl, 25 mM./kg. in two to four hours in an attempt to eliminate the deficit and also to provide for growth. However, these survived less than four hours after the initiation of treatment and therefore are included in the potassium-deficient control group. In all of the groups, a few animals were used for carcass analysis and were not available for complete histological examination. The accompanying tabulations summarize the data on animals on which pathological examination was performed.

The Diet

The composition of the potassium-deficient diet was as follows:

Dextrose	505 gm.	Nicotinic acid	40 mg.
Corn oil (Mazola®)	150 gm.	Calcium pantothenate	20 mg.
Washed casein (vitamin-free)	300 gm.	Choline hydrochloride	1.0 gm.
Thiamine hydrochloride	4 mg.	Haliver oil	2 cc.
Riboflavin	8 mg.	Salts 4 (modified as below)	42.3 gm.
Pyridoxine hydrochloride	4 mg.		

The salt mixture [4] contained the following constituents in grams per kilogram of the diet:

$CaCO_3$	13.5	Fe citrate.$6H_2O$	1.237
NaCl	7.52	NaF	0.03
Na_2HPO_4	11.9	$MnSO_4.4H_2O$	0.226
$CaHPO_4.2H_2O$	3.37	$CuSO_4.5H_2O$	0.0135
$MgSO_4.7H_2O$	4.59	$ZnCl_2$	0.0112

The diet was shown to contain electrolytes in millimols per kilogram as follows:

Ca	154	P	108
Na	295	Cl	135
Mg	18.5	K	Less than 1

3. This was "purina® chow checkers," made by the Ralston Purina Company, St. Louis.

4. Hegsted, D. M.; Mills, R. C.; Elvehjem, C. A., and Hart, E. B.: Choline in the Nutrition of Chicks, J. Biol. Chem. **138**:459, 1941.

The diets were mixed as follows: The salt components were first weighed out, ground to a powder and mixed, and sifted. They were then added to the weighed casein and dextrose, and these were thoroughly sifted and mixed dry. The water-soluble vitamins were dissolved in 25 cc. of 50% ethanol, and the solution was sprayed or added drop by drop to the dry mixture, which had been spread out on flat pans. After drying, the powder was again sifted and mixed. The haliver oil was added to the corn oil, and this mixture was rubbed into the powder until

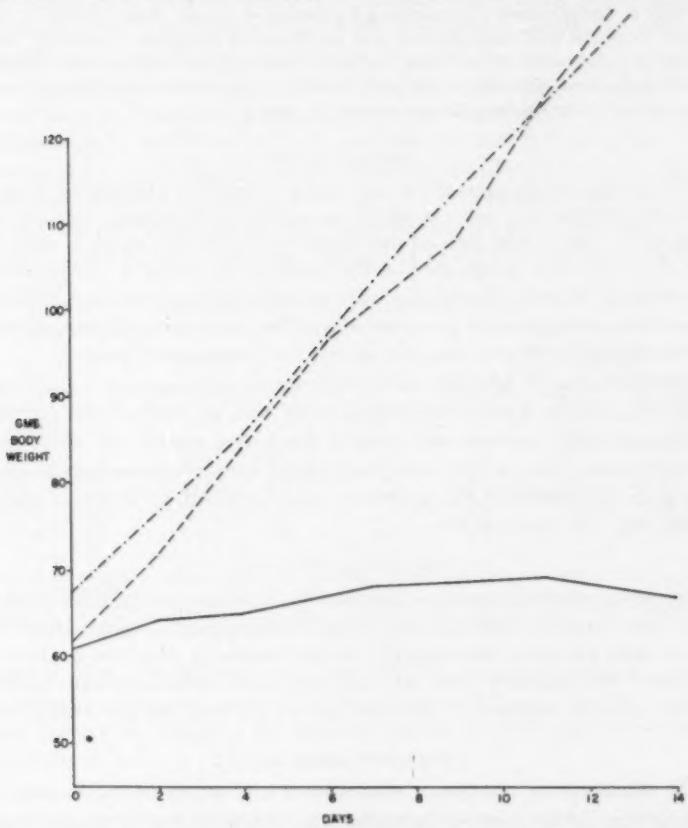

Fig. 1.—Growth curves: controls, fed a laboratory chow (- - - - -); experimental rats, fed a potassium-deficient synthetic diet (————); experimental control rats, fed the synthetic diet supplemented with potassium bicarbonate (· — · — · —).

a homogeneous paste was obtained. Because of the observations of Thomas, Mylon, and Winternitz [5] and Follis,[6] concerning the dependence of the effects of potassium deficiency on thiamine hydrochloride, approximately 10 mg. to the liter was added to the drinking water, which was given ad libitum.

5. Thomas, R. M.; Mylon, E., and Winternitz, M. C.: Myocardial Lesion Resulting from Dietary Deficiency, Yale J. Biol. & Med. **13**:523, 1941.

6. Follis, R. H., Jr.: Myocardial Necroses in Rats on a Low Potassium Diet Prevented by Thiamine Deficiency, Bull. Johns Hopkins Hosp. **71**:235, 1942.

The diet was mixed fresh every three weeks, and the animals were fed in three separate groups. Dietary controls were present in each group. Where the control diet was the deficient diet supplemented by potassium, potassium bicarbonate, 8.34 gm. per kilogram of diet, was added to the salt mixture.

The animals were killed by exsanguination under ether anesthesia, the skeletal muscle was sampled for chemical study, and an immediate complete autopsy was performed. The soft tissues were fixed in Zenker's acetic acid solution and embedded in paraffin. Knee joints were fixed in formalin, decalcified in 5% aqueous nitric acid, and embedded in celloidin (pyroxylin). Sections were stained with hematoxylin and eosin, Mallory's aniline blue, and reticulum stain as indicated. Detailed studies were made only of the heart, skeletal muscle, and knee joint sections, although several sections of the remaining viscera were also examined.

WEIGHT CURVES

The average weight gains of the rats on the control diet (laboratory chow), the potassium-deficient diet, and the potassium-supplemented deficient diet are shown in Figure 1. The weight gain on the supplemented diet was equal to that on the chow diet. The daily weight gain on the deficient diet averaged 1.10 gm. per day up to the peak weight, while the same animals, during the preliminary control period on the chow, averaged 4.98 gm. gain daily. The potassium-supplemented dietary controls averaged 5.06 gm. gain daily during the experimental period.

Potassium chloride administered by injection in solutions with a concentration of 100 mM./liter in doses corresponding to 17 mM./kg. and 25 mM./kg. caused weight gains which approximated those of the control animals but which stopped after three days. These weight gains were delayed when potassium acetate solutions were used. The details of the reparative weight gain will be presented elsewhere together with the chemical data.

INCIDENTAL FINDINGS

The gross intestinal distention described by Kornberg and Endicott [7] and Henrikson [8] was seen in a large majority of the deficient animals. In the animal whose kidneys were examined histologically, tubular lesions as described by Follis and associates [2] were regularly seen, but these two types of alteration were noted incidentally, since the emphasis of the study was on the heart and the skeletal muscle.

PATHOLOGIC OBSERVATIONS

The accompanying table lists the numbers of rats among those examined which had lesions in cardiac muscle, skeletal muscle, and epiphyseal cartilage. Since the incidence of each of the pathologic changes differed according to the number of days the rats had subsisted on the deficient diet, grouping of the animals in the table was devised on the basis of time. Each listing, therefore, includes similar numbers of animals from each reparative experimental group. No differences in the degree of pathologic change could be detected, whether 17 mM./kg. of potassium chloride, or 25 mM./kg. of KCl, or 25 mM./kg. of potassium acetate was given.

7. Kornberg, A., and Endicott, K. M.: Potassium Deficiency in the Rat, Am. J. Physiol. **145**:291, 1946.

8. Henrikson, H. W.: Effect of Potassium Deficiency on Gastrointestinal Motility in Rats, Am. J. Physiol. **164**:263, 1951.

Cardiac Muscle.—The cardiac lesions were identical with those described by Follis, Orent-Keiles, and McCollum.[2] Gross changes were seen in only a few animals and consisted of small, patchy, irregular gray areas. Microscopically, the typical loss of cross striation followed by disappearance of sarcoplasm or by necrosis of the muscle fibers occurred in nearly all the animals. Cellular collections, consisting mainly of mononuclear phagocytes, occupied either small, patchy areas or infiltrated diffusely into large portions of the myocardium, especially in the subendocardial region and the papillary muscles. Necrosis of cardiac muscle fibers was seen only infrequently, and not extensively. Polymorphonuclear leucocytes also were infrequently found and usually in relation to necrotic fibers. While a few fibroblasts could be seen in the lesions, most of the cells present were mononuclears.

During the period following replacement of potassium, up to one week, the deposition of connective tissue fibers in the cardiac lesions could not be demonstrated in quantities greater than were present in the rats of the control deficiency period. The muscle fibers adjacent to or coursing through the cellular lesions did not show

*Lesions in Potassium Deficiency and Repair**

Days of Deficiency	Rats	Days After Treatment	Muscle K†	Plasma K†	Number of Rats with Lesions Among Number Examined		
					Cardiac Muscle	Skeletal Muscle	Epiphyseal Cartilage
14	21	0	33.0	1.5	17/21	15/17	1/15
15	9	1	42.5	3.17	9/9	5/6	2/6
16	7	2	35.5	2.92	6/7	0/3	3/3
18	5	4	32.0	1.90	5/5	1/3	3/3
19	3	5	31.1	2.0	3/3	0/3	3/3
20	2	6	34.5	2.0	2/2	1/2	1/2
21	3	7	24.8	1.63	2/2	1/1	1/1

* This is a tabulation of the numbers of rats whose tissues were examined histologically at various stages of the experiment.

† Muscle K is expressed as millimols per 100 gm. of fat-free dried tissue; plasma K, as millimols per liter. The first group of 21 rats includes those which died during the 14-day deficiency period. The control animals showed a muscle K of 49.0 mM. and a plasma K of 4.24 mM. per 100 gm. of fat-free solids.

any nuclear changes or clubbing of the ends which might be interpreted as evidence of regenerative phenomena. In fact, no histological differences could be made out between the cardiac muscle of the deficient rats which underwent treatment and those which did not.

Skeletal Muscle.—The skeletal muscle lesion was demonstrable grossly in only four animals. In these it appeared as a change of color and consistency which either involved entire muscle groups or diffusely affected the skeletal muscle of the entire animal. The hind legs showed the greatest involvement, in the few animals in which the change was grossly detected. The muscles appeared pearly gray or were speckled red and gray and were of firmer consistency than usual. Microscopically (Figs. 2, 3, and 4), lesions were present in nearly all the deficiency animals and, for the most part, the changes were those of Zenker's degeneration. The muscle fibers first lost their cross striation and then either underwent necrosis or, more frequently, lost their sarcoplasm. This loss of sarcoplasm usually involved large groups of fibers in an area, but occasional fibers could be seen in which portions of the sarcoplasm were fragmented and stained poorly.

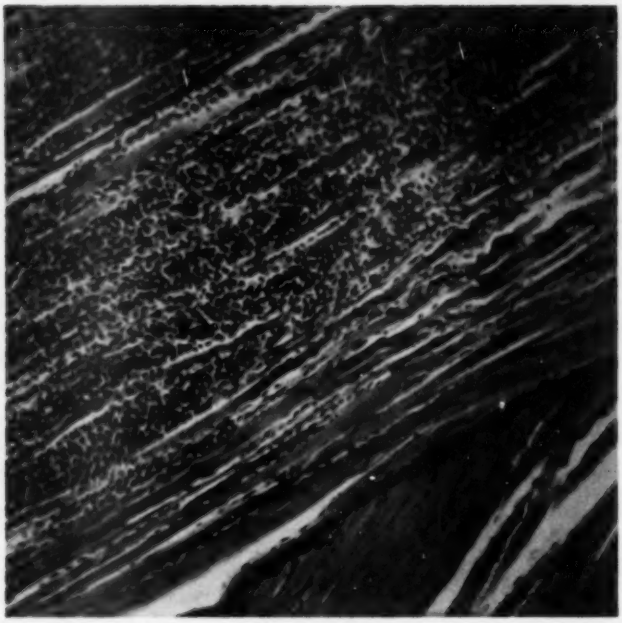

Fig. 2.—There is extensive destruction of muscle fibers, with lost fibers being replaced by mononuclear and polymorphonuclear cells. Note the severe involvement of one muscle area and the lack of involvement of the adjoining area (lower right).

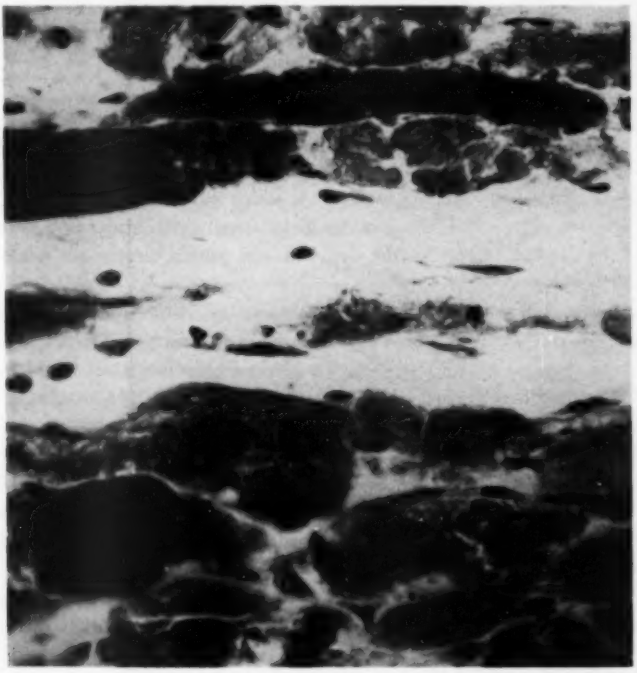

Fig. 3.—The dissolution of the sarcoplasm of the central fibers is evident. Necrosis of a few fibers and loss of cross striation also can be seen.

In the animals in which gross lesions were present, nearly all fibers were involved to some extent by the degenerative process. In these animals, and in occasional patches of muscle of animals with no gross lesions, the process had advanced beyond loss or necrosis of sarcoplasm. Here the sarcolemmal nuclei had survived and could be seen lining the sarcolemmal tubes. Each nucleus was bordered by a small amount of cytoplasm, and where the cells were in apposition their syncytial character seemed to persist. While a few of the sarcolemmal tubes remained empty, most of them were occupied by varying numbers of mononuclear phagocytes, while several, especially

Fig. 4.—The regeneration of muscle fibers is evident after potassium replacement. Mitotic figures are common. The syncytial nature of the regenerative fibers also can be seen. The large fibers which survived the period of deficiency are separated by thinner, regenerating fibers.

where necrotic fibers were in evidence, contained polymorphonuclear leucocytes in moderate numbers.

All stages of repair of the skeletal muscle lesion could be seen. The repair was extremely rapid and in two days regeneration had nearly obliterated the lesions in some animals. The manner of repair, for the most part, consisted of reconstitution of the fibers by multiplication and production of sarcoplasm on the part of the sarcolemmal syncytial cells. Mitoses were common, and fibers in all stages of regeneration could be seen. An increase in the amount of stainable collagen was not evident despite the presence of numbers of fibroblasts, particularly in the areas where necrotic fibers occurred.

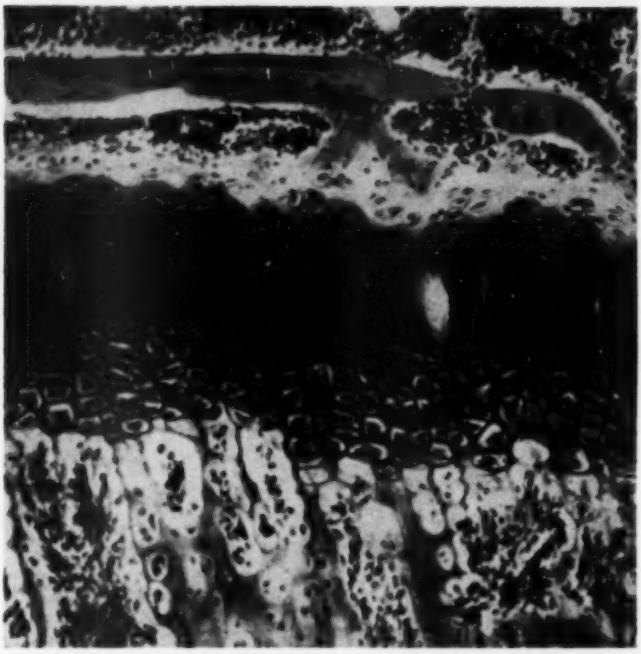

Fig. 5.—Here is seen the earliest phase of cartilage degeneration. The fibrillar matrix in the proliferative cell zones and the loss of matrix in the "hypertrophic cell zone" are to be noted.

Fig. 6.—Further degeneration and loss of the matrix is evident. The hypertrophic cell zone is thin, while the proliferating cells are compressed.

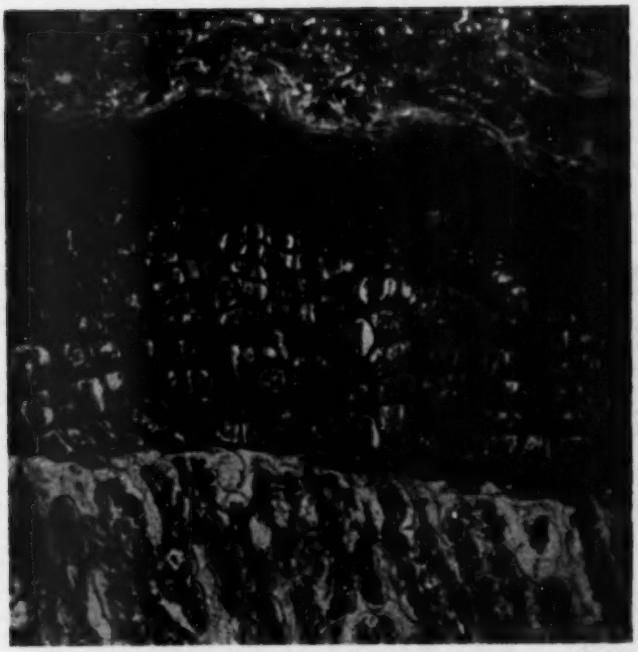

Fig. 7.—Only a small amount of cartilage matrix remains. The columnar arrangement of the cartilage cells has not been disturbed, and the proliferative zone is compressed.

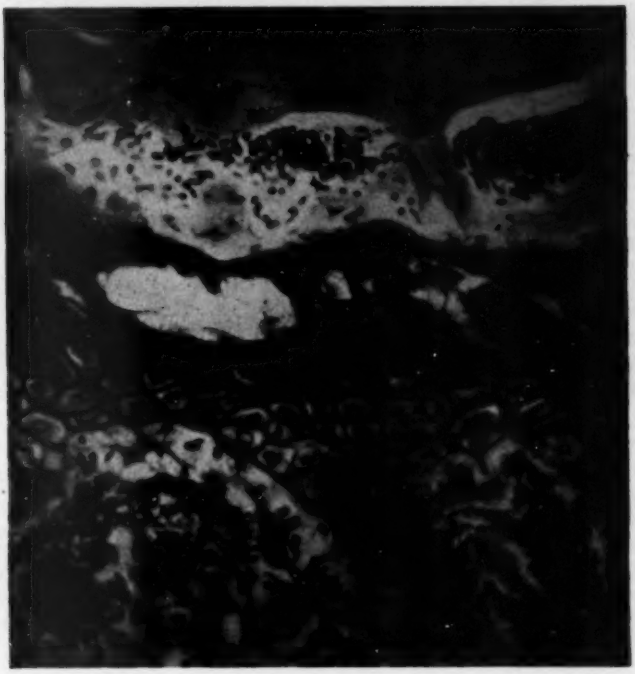

Fig. 8.—The proliferative zone has been torn in the region of cartilage degeneration. This is the latest phase of the cartilage lesion seen in this series.

Epiphyseal Cartilage.—The lesions of epiphyseal cartilage did not occur regularly before the 16th day of the experiment. Thereafter, the lesion was progressively severer (Figs. 5, 6, 7, and 8). Both matrix and cellular changes were evident. The earliest sign of the lesion in the matrix was the occurrence, in the proliferative cell zone, of small areas of degeneration of the cartilage matrix in

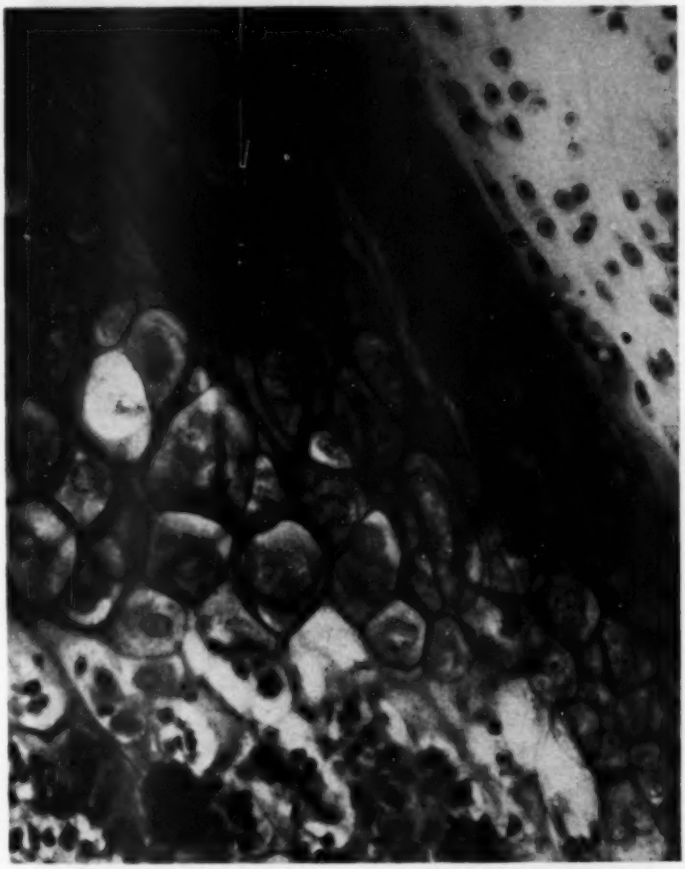

Fig. 9.—The border of the epiphyseal cartilage is shown here (perichondrial ring). Mitotic figures are evident, and cartilage degeneration as well.

which there occurred some loss of staining reaction and some fibrillation. At first the areas were small and scattered, but in the later lesions practically each column of cells in the proliferative zone was separated from the neighboring columns by areas of degeneration. Still further progress of the lesion led to the disappearance of nearly all cartilage matrix below the zone of indifferent cells ("reserve" cell zone of Dodds and Cameron), and occasionally horizontal clefts were produced in this

region by sectioning trauma despite celloidin embedding. This can be attributed to the weakening of the area by the loss of matrix. Cytological changes were visible in the cartilage of the epiphyseal plate and to a less degree in the articular layer also. The zone of indifferent cells was decreased in thickness, and the individual cells were larger, rounder, and better demarcated than usual. The nuclei were not as flat as in normal cartilage and showed intensified basophilia. The cytoplasm was increased in volume and stained poorly. The zone of proliferating cells was somewhat decreased in thickness and was composed mainly of cells near maturity, i. e., only a few paired and four-celled groups were found, compared with the plentiful large square cells. In the less mature portion of this zone numerous mitotic figures could be seen (Fig. 9) both in the perichondrial ring and in the periphery of the epiphyseal plate. The central part of the epiphyseal plate and the articular cartilage layer did not contain mitotic figures. The zone of hypertrophic cells was thickened to double or triple its usual width but retained its orderly columnar arrangement. The cells were very large and contained much clear cytoplasm. Between the columns of cells the matrix was very scant. Those cells nearest the marrow cavity, in a layer three or four cells in thickness, showed a loss of nuclear and cytoplasmic chromatin and were swollen to nearly double the usual size.

In addition to the lesions of epiphyseal cartilage described, nonspecific changes of the ends of the bones were found which were attributed to inanition. They consisted of a diminution of the number and thickness of trabeculae in the primary and secondary spongiosa and a slight thinning of the cortical bone and also of the entire epiphyseal cartilage plate. No changes of note were detected in the soft tissue components of the knee joint. The bone marrow often showed intense hyperemia, with hematopoietic tissue replaced by dilated vessels, but no disturbance of hematopoietic sequences could be made out.

In contrast to the rapid repair of the lesions of skeletal muscle, those of the epiphyseal cartilage showed no signs of return to the normal. Since no animals were maintained on the pure deficient diet beyond 14 days, and in that period the cartilage lesion did not become evident, a comparison of animals with and without the one-day potassium therapy was not possible. Therefore, the significance of the therapy in regard to the production of these lesions cannot be evaluated with the data at hand.

COMMENT

Two questions concerning the lesions described arose in evaluating their significance: (1) How did they compare with lesions of muscle and cartilage produced by other nutritional deficiencies, and (2) what relationship did the lesions bear to potassium deficiency?

When dietary deficits which produced lesions of skeletal muscle were sought for in the literature, the deficiency of vitamin E provided the outstanding instance. A comparison of the lesions of skeletal muscle described in these experiments and those produced in weanling rats by diets deficient in vitamin E showed three essential points of difference.

First, the duration of dietary deficiency of potassium needed to elicit the lesions was about two weeks, while that of vitamin E deficiency was a few months. Second, the lesions from potassium deficiency were very large and sometimes generalized,

while in vitamin E deficiency small, focal, disseminated lesions were the rule. Third, repair of these lesions when potassium was administered was extraordinarily rapid, while repair of the muscle lesions of vitamin E deficiency was rather slow. The essential histologic character of the lesions compared with those of vitamin E deficiency was not dissimilar if one took into account the differences in rate of production and repair. Both were primarily degenerations of muscle similar to Zenker's degeneration. The lesions in this series of experiments were not followed long enough to determine whether healing by fibrosis would occur. Healing by regeneration of muscle fibers occurred, as it did in vitamin E deficiency. The diet here used provided adequate vitamin E in the corn oil fraction. While muscle lesions have been described in other animal species as being produced by diets deficient in other factors than those referred to—e. g., ascorbic acid, "anti-stiffness factor"—they were not considered germane to the present study.

Specific lesions of the epiphyseal cartilage of the rat are found in three dietary deficiencies: vitamin D, vitamin A,[9] and ascorbic acid [10] deficiency. Nonspecific changes are frequently found associated with malnutrition or systemic disease. The lesion described in this study bore no resemblance to any of the above-mentioned specific pathologic entities, nor has it been seen in the severest inanitional states. While small foci of degeneration of the matrix of epiphyseal cartilage can be demonstrated occasionally in routine animal preparations, widespread degenerative lesions of the cartilage matrix have not been described in the rat heretofore. Additional studies of these lesions are being carried out to clarify specifically whether the lesion arises in response to the potassium therapy as administered or can be produced by the deficient diet without supplementation.

The answer to the question whether these lesions of muscle and cartilage were due to pure deficiency of potassium cannot be given conclusively. The concomitant lesions of heart, intestine, and kidney, previously described in rats on potassium-deficient diet, provide good evidence that severe potassium deficiency was present. The chemical studies, reported elsewhere, were confirmatory. However, the inability of other investigators of potassium deficiency to demonstrate these lesions of muscle and cartilage must be cited. Differences in the diets used may be responsible for differences in the results. The biochemical sequences of development of any of the lesions mentioned are not known, and the manner of the evolution initiated by dietary electrolyte deficits remains to be demonstrated.

A scrutiny of the diets used in these experiments and in published studies concerned with potassium deficiency does not reveal any single difference upon which the discrepancy of results can be explained. It is well known that the relative proportions of various factors in the diet can be as important as their absolute concentrations. The interrelation of potassium, magnesium, and protein is one example, which may be pertinent to this study, where deficiency of one factor may lead to

9. Wolbach, S. B.: Vitamin-A Deficiency and Excess in Relation to Skeletal Growth, J. Bone & Joint Surg. **29**:171, 1947.

10. Wolbach, S. B., and Maddock, C. L.: Cortisone and Matrix Formation in Experimental Scorbutus and Repair Therefrom, A. M. A. Arch. Path. **53**:54, 1952.

relative deficiency of others.[11] In the diet used, the content of casein and of sodium should be noted. The casein fraction provided somewhat more protein than is usual in stock diets. This was designed to prevent protein deficiency because of diminished intake. The sodium concentration was purposely elevated to accelerate the development of potassium deficiency. The effects of increased sodium intake therefore must be considered in addition to the potassium deficiency effect. A separation of the effects of these two factors may prove of interest in subsequent experiments.

SUMMARY

Rats fed a diet containing less than 1 mM. of potassium per kilogram of body weight for two weeks showed the typical cardiac, gastrointestinal, and renal lesions of potassium deficiency and showed widespread muscle lesions as well. These resembled Zenker's degeneration. One day's therapy with parenteral potassium solution caused rapid repair of the muscle lesions, but after a few days characteristic degenerative lesions of epiphyseal and articular cartilage were found. The cardiac lesions showed no signs of repair under these conditions.

11. Colby, R. W., and Frye, C. M.: Effect of Feeding High Levels of Protein and Calcium in Rat Rations on Magnesium Deficiency Syndrome, Am. J. Physiol. **166**:408, 1951. Cannon, P. R.; Frazier, L. A., and Hughes, R. H.: The Influence of Potassium on Tissue Protein Synthesis, Metabolism **1**:49, 1952.

DORSAL INTESTINAL FISTULA; ACCESSORY NEURENTERIC CANAL; DIASTEMATOMYELIA

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IN THE YEAR 1906 Keen and Coplin [1] described in a healthy 2-year-old girl a narrow open tubular tract extending from the dorsal wall of the rectum to the body surface through the skin in the midline of the lower sacral region (Fig. 1*a*). Probably because of the valve action of some mucosal fold, no fecal matter escaped during defecation, but a few drops of fluid could be expressed after the administration of an enema. At operation the tract was found to pass through a median defect in the bodies of the fused lower sacral vertebrae, between the nerve roots of the cauda equina, and, in the outer third of its course, through a fatty subcutaneous tumor over which the skin showed a slight overgrowth of hair. The tube measured 4 by 5 mm. for most of its length, but narrowed as it opened through the skin. Microscopically, its wall resembled that of a bronchus. This type of anomaly might be called, purely descriptively, a dorsal intestinal fistula.

In considering the embryonic conditions which may throw light on this anomaly, one is reminded of a somewhat similar normal opening from the yolk sac cavity (the future intestinal cavity) to the then surface of the embryo within the amniotic cavity, the neurenteric canal. The term "neurenteric canal," implying an open passage between the enteric cavity and the neural groove, is applied in vertebrate embryology to a minute short-lasting tube running from the yolk sac cavity through the primitive knot (Hensen's node) to the amniotic cavity. It is probably homologous with the blastopore or primitive mouth of some early form such as the polyp. In man the canal develops in relation with the formation of the notochord and hence has been also called the chorda canal. The process in a presomite human embryo is described by Heuser.[2] The primitive knot serves as a center of cell proliferation and later gradually becomes unrecognizable because of the dispersal of the newly formed cells. The position of its remains in later fetal life can, however, be fairly accurately located. Since the newly differentiated tissues from the knot and from its attendant primitive streak (neural ectoderm, mesodermal somites) migrate cranially, the remains of the knot should be sought at the caudal ends of their derivatives, the spinal cord and the vertebral column. The retraction of the tail with the loss of both its spinal cord and its vertebrae leaves the much diminished knot at the tip of the coccyx, and there it may be supposed to remain in spite of the further

From the Department of Pathology, The Children's Hospital.

1. Keen, W. W., and Coplin, W. M. L.: Sacrococcygeal Tumor, Surg., Gynec. & Obst. **3**:662-671, 1906.

2. Heuser, C. H.: Presomite Embryo with a Definite Chorda Canal, Contrib. Embryol. **23**:251-267, 1932.

loss of the three lower coccygeal nerves and the late fetal migration of the spinal cord up the spinal canal caused by the more extensive growth of the latter. Perhaps the prevalence of teratoma in this region may be due to the presence of polyvalent cells remaining from the primitive knot, and again perhaps the pilonidal sinus may represent the much exaggerated outer (ectodermal) end of the neurenteric canal. These are unsubstantiated guesses. The main point lies in the fact that the coccygeal tip is the final location of the reduced and normally unrecognizable primitive knot and the former neurenteric canal, and that any open connection between the intestine and the dorsal surfaces in a region cranial to the coccyx must be an ectopic or an accessory canal.

Such dorsal intestinal connections have, in fact, been produced experimentally in vertebrae embryos. In 1868 Lereboullet [3] after artificial insemination of fish embryos (pike), found among many normal individuals a few in which the embryo, while still flat on the globular yolk, showed surprising features. The head end and the tail end of the embryo were normal, but along the greater part of the body the embryo was split sagittally, the two halves spreading widely apart to expose between them the upper surface of the yolk (Fig. 1*b*). In 1888 Roux [4] was able to produce a similar condition in the frog from the remaining cell after destruction of one of the first two daughter cells (blastomeres) of the fertilized ovum. The embryos, however, were incomplete in that they lacked a part or all of the caudal portion of the body. He called this condition asyntaxis or diastasis medullaris. Four years later Hertwig [5] produced the same type of malformation, also in the frog, by delaying the union of the spermatozoa and the mature ova or, as he describes it, by the overripeness of the ova. His figures (Fig. 1*c* and *d*) show a large mound of the yolk bulging between the two widely separated halves of the body of the embryo. For this condition Hertwig employed the term "diastematomyelia" (in Greek, the word "diastasis" means a separation, and "diastema," a cleft or fissure).

Both of these terms refer particularly to the spinal cord. Actually in the malformed embryos of Lereboullet and the later anatomists the cleft affects not only the neural groove, representing the future spinal cord, but also the notochord, the two halves of which can be seen on either side of the protruding yolk mass (Fig. 1*c* and *d*) and the dorsal wall of the yolk sac cavity, the future intestine. In other words, the wide-open cleft in these embryos has all the characteristics of a neurenteric canal but differs from its early embryonic prototype in size and in position, since it is far craniad to the caudal end of the spinal canal. As neurenteric canals or blastopores, Hertwig accepted these clefts, as the title of his chief paper on this subject indicates—"Urmund und Spina bifida."

Somewhat similar openings from intestine to dorsal surface have been noted in man. A very few cases have been reported (I have found three in the literature) referring to different parts of the intestinal tract. In one [6] there was a broad connection between the pharynx and the esophagus and the wide open cervical and thoracic

3. Lereboullet: Ann. sc. nat., Series 4 (Zoology) T. 20, 1868.

4. Roux, W.: Ueber das künstliche Hervorbringen halber Embryonen, Arch. path. Anat. **114**:113-153, 1888.

5. Hertwig, O.: Urmund und Spina bifida, Arch. mikr. Anat. **39**:353-503, 1892.

6. Muscatello, G.: Über die angeborenen Spalten des Schädels und der Wirbelsaüle, Arch. klin. Chir. **47**:162-301, 1894.

cord; in one [7] the stomach and intestines opened at several points along the back onto the floor of a large myelomeningocele with rachischisis, the thin roof of which had been torn at birth. The third case, reported in the same paper, showed a single open tract from the esophagus to the floor of an opened myelocele, with an associated encephalocele above and a wide-open spinal cord below, due to the failure of intrinsic cord development beyond the three-week stage of the embryo. All three were found in stillborn infants.

These three specimens in which the wide tract opened between the separated halves of the spinal cord closely resembled the experimental embryos of Hertwig

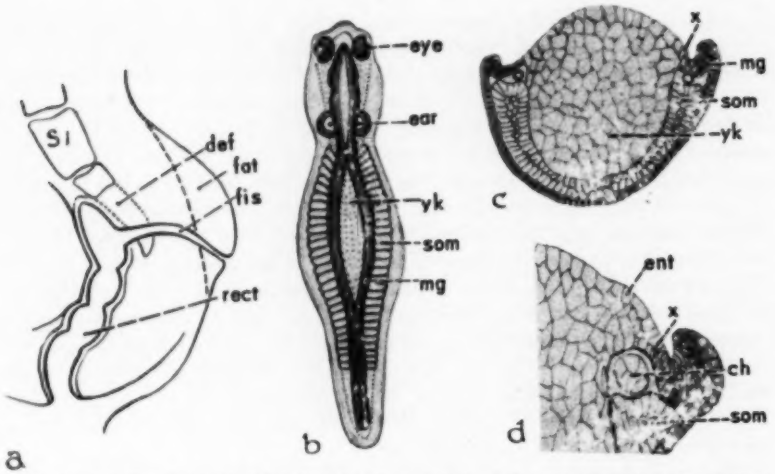

Fig. 1.—(*a*) Dorsal intestinal fistula (after Keen and Coplin [1]). (*b*) Anomalous pike embryo, (Hertwig after Lereboullet [8]). (*c*) and (*d*) Sections of anomalous frog embryos (Hertwig [8]). Key to abbreviations: *ch*, notochord; *ent*, entoderm; *def*, vertebral defect; *fis*, dorsal enteric fistula; *mg*, medullary groove; *rect*, rectum; *som*, somite; *yk*, yolk; *x*, junction of ectoderm and entoderm.

and others and may be considered as representing accessory neurenteric canals. They and the experimental embryos all differ from the anomaly of Keen and Coplin, already described, in that in the latter the tract passes from intestine to skin. The tract has been lengthened by the distance between the ventral commissure of the spinal cord and the dorsal surface.

In the normal development of fish and amphibia, the yolk granules, at first loading all the yolk cells, are gradually absorbed from the more dorsal region of the yolk, leaving a simple yolk-free cell layer, the entoderm, as the roof or dorsal wall of a fluid-filled cavity. Such a layer over the yolk mass seems to be in the process of formation in the older of Hertwig's sectioned embryos (Fig. 1*d*, *ent.*). In mammals, with their minimal yolk mass, the fluid space and entodermal layer appear much earlier, and as the two sides of the split embryo grow closer together in the process of repair, the fluid space will be laterally compressed, forcing its new roof to bulge

7. Lucksch, F.: Über Myeloschisis mit abnormer Darmausmündung, Ztschr. Heil. **24**:143-156, 1903.

and protrude between the separated body halves as a hollow tube or longitudinal ridge, which might finally meet and open through the skin of the back, or might be partially or wholly obliterated by the final meeting of the halves. If it remains patent, the vertebrae and other median structures must grow around it. This sequence is suggested in the diagram (Fig. 2). As the embryo grows larger the tube must grow longer, and to provide for its increasing lining epithelium may draw on the adjacent surface layer, with the result that sometimes the outer portion of the fistula may be lined by turned-in fetal skin, and when isolated may assume the form of a dermoid cyst or tumor.

A dorsal intestinal fistula may well remind one of another, better known type of intestinal fistula, the umbilical fistula, or the patent form of Meckel's diverticulum. In position and developmental origin these two have nothing in common; their sole similarity lies in the fact that both are open passages extending from the intestinal tract to the surface of the body. As such, it is entirely possible, even probable, that

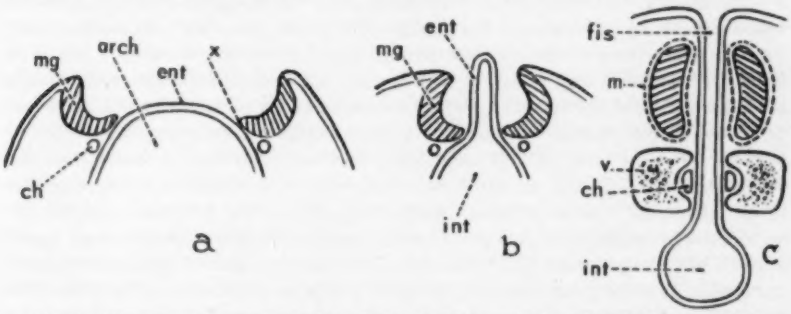

Fig. 2.—Diagrammatic representation of the probable method of lengthening the tract as the neural groove closes to form the neural tube. (*a*), (*b*), and (*c*) show increasingly older stages. Key to abbreviations: *arch*, archenteron; *ch*, notochord; *ent*, entoderm; *fis*, fistula; *int*, intestine; *m*, meninges; *mg*, medullary groove; *x*, junction of ectoderm and entoderm.

the known variations in form common in the one may also be found in the other. Meckel's diverticulum rarely remains as an open fistula; normally the tract disappears without leaving a trace. Between these two extremes, its former presence may be indicated by a fibrous cord running from intestine to umbilicus or joining with a still patent portion of the tract, which may open at the umbilicus or into the intestine, or appear as a closed cyst anywhere along the cord. Finally, the fibrous cord may be lost in part or completely, leaving the cyst either attached to one end of the original tract or completely unattached. Many of these variations of the diverticulum are well known as surgical possibilities and may be expected also in dorsal intestinal fistulae. Meckel's diverticulum runs simply through the body (peritoneal) cavity; the similar fistula to the back must take a more complicated course through the midline notochord and neural groove in the young embryo, and after the mesodermal components have swept medially from the somites to form the vertebrae and the soft tissues of the back, through all the structures in the median line, or between their respective halves, if they remain apart. It is among these various tissues and structures that the cystic variations may be found.

Many such cysts of dorsal fistular origin have probably been mistaken for meningoceles, either dorsal or ventral (anterior), and others, in the lower cervical or

mediastinal region, for branchial cysts or the tips of supernumerary bronchi. The character of the wall of the cyst, while marking the meningocele by its flat epithelium and lack of muscles, is not of much help in distinguishing between the two latter groups, for the epithelium, though usually copying that of the parent intestinal level, may retain its fetal form or may have become flattened by the pressure of the enclosed fluid. The one sure indication that the cyst is of fistular origin is the presence of a tubular tract or fibrous cord leading from the cyst or other remnant to some median structure; the finding, in median line structures, of recognizable proof of the former separation and later reunion of their parts, even although no present connection with the tract can be demonstrated, is strong presumptive evidence.

A few instances of incomplete dorsal fistulae have been noted, although not as such, in the records of this hospital. In one case the open outer end of the fistula was found near the dorsal midline in the lumbar region, and from it constantly oozed a nonfecal fluid, the product of its own epithelium. At operation a fibrous cord was found connecting it to the spinous process of a lumbar vertebra. In another case, a projecting mound in the midthoracic region proved to be caused by a lobule of partially expanded lung, complete with alveoli and a small bronchus which could be followed to the underlying spinous process where it ended. Such a condition could well be the result of a former fistula extending from the surface to the pharynx at the level of the lung bud and including a portion of the lung primordium in the wall of the tract. In still another case, a large cyst, discovered by roentgenograms taken because of a wide deviation of the esophagus to the left, was mistaken for an anterior meningocele. At operation the spinal cord and meninges were found normal, but a large medial defect was noted in the body of one of the lower thoracic vertebrae. A subsequent operation revealed a large retropleural cyst bulging from the dorsomedian wall of the right pleural cavity and attached to a fibrous cord leading toward the vertebral defect. The wall of the cyst resembled that of the fetal esophagus. The direction taken by the attached fibrous cord, together with the presence of the vertebral defect, serves to differentiate the origin of this cyst from that of a group of other possibilities.

Cases showing proof that the midline structures formerly were separated, presumably by a dorsal intestinal fistula, and that they subsequently attempted to unite when the fistulous tract had shrunken or disappeared, are more numerous. They do not include cases of spina bifida or of dorsal meningocele, for the processes by which the wide open neural groove is transformed to the closed neural tube and then separated from the skin ectoderm, thus allowing space between them for the mesenchyma in which the paired spinous processes are differentiated, are all a part of normal growth, and the conditions mentioned are merely instances of incomplete development. On the other hand, the vertebrae and the spinal cord are exactly the structures most affected in the condition under discussion.

In the vertebrae the former presence of the dorsal midline fistula is shown in three different forms: If the fistulous tract is lost soon after its formation, additional bone may fill the gap, causing merely increased breadth of the vertebral body or bodies; or, if the fistula persists longer, the bodies of one or more vertebrae may develop around the already present tract, enclosing it in a circle or oval of bone, so that in the later disappearance of the fistula there is left an empty defect or hole through the vertebral body; or perhaps in the case of a larger fistula, the

two half bodies may grow as individuals, each developing both right and left pedicular processes. In the last case, when the two bodies later come together, the contiguous processes fuse to form a median spur, bony or cartilaginous, projecting into the spinal canal. If any fragment of the fistulous tract remains (as, for instance, a fibrous cord attached to an inconspicuous midline skin dimple), it may enter the tip of this spur. Dorsally the two meeting spinous processes may fuse abnormally. In all three forms the spinal canal will be widened locally.

In the spinal cord the condition known as diastematomyelia supplies ample proof of the former presence of a dorsal intestinal fistula. The two halves of the cord have been separated locally in a widened spinal canal, often accompanied by the median ventral bony spur just mentioned or by the vertebral defect. In the spinal cord, however, there is little attempt to close the cleft between the two separated halves. Perhaps the halves are restrained by the fixation of their nerve roots, ventral and dorsal. The ever-spreading mesenchyma grows between and around them, and from it each half cord is enclosed by a meningeal investment, the two median walls of which meet in a fused layer of dura or arachnoid. In this fused layer any remnant of an earlier fistula, fibrous cord or cyst, may be found. Within this investment each half of the neural groove continues its normal development as best it can, either as the lateral half of a spinal cord (diastematomyelia) lacking, of course, the normal commissures, or as a pair of malformed whole cords (diplomyelia [8]), each half attempting to complete the normal whole just as the half vertebral bodies attempt to form both pedicular processes. The two conditions often go together and the median spur, penetrating between the two contiguous dural leaves, is a common finding in diastematomyelia.[9] The two factors, indications of the former medial cleft both in spinal cord and in bone, make a complete picture, and offer definite proof of the earlier presence of a dorsal intestinal fistula.

Both factors, however, are not always evident. In the latter half of fetal life and the early postnatal period, the spinal cord normally "migrates" up the spinal canal. The injured level of the cord may thus travel a considerable distance craniad to the injured bone, though both injuries were caused by the same fistulous tract. The widened cord may be but slightly compressed in the more cranial position, for the normal cord is loosely suspended, with plenty of unused space between it and the walls of the canal. The local absence, in diastematomyelia, of all spinal cord commissures may pass unnoticed because of the abundant connections of the nerves affected with others above and below. The condition would remain hidden and unsuspected, discoverable only by myelography following the injection of some radiopaque substance to bring out the local doubling of the arachnoid space.[9a] Yet the presence of the bony injury alone is strong presumptive indication of the true condition.

In the presence of a bony spur long enough to become engaged between the leaves of the fused dural membranes or even to divide the vertebral canal locally into two compartments, full craniad migration of the cord is effectively prevented.

8. Herren, R. Y., and Edwards, J. E.: Diplomyelia (Duplication of Spinal Cord), Arch. Path. **30**:1203-1214, 1940.

9. (*a*) Neuhauser, E. B. D.; Wittenborg, M. H., and Dehlinger, K.: Diastematomyelia: Transfixation of Cord or Cauda Equina with Congenital Anomalies of Spine, Radiology **54**:659-664, 1950. (*b*) Matson, D. D.; Woods, R. R.; Campbell, M. D., and Ingraham, F. D.: Diastematomyelia (Congenital Clefts of the Spinal Cord), Pediatrics **6**:98-112, 1950.

The spur is found at the caudal end of the cleft in the cord, and the pressure it exerts on the commissures of the normal cord below is a measure of the tension of the cord above. The physical symptoms found in this type of diastematomyelia and their relief on removal of the spur are described by Ingraham and his group.[9b] The two injuries, to the bone and to the spinal cord, are at the same level and offer double proof of the former presence of a dorsal intestinal fistula, even in the absence of any remaining sign of its presence.

SUMMARY

The dorsal intestinal fistula is a definite entity, somewhat similar in its course to the normal, short-lasting neurenteric canal extending from the yolk sac to the amniotic cavity through the primitive knot. Experimentally, anomalous embryos of fish and amphibia have been produced, showing a normal head and tail end but with the back between separated into two halves by a long median cleft, through which the underlying yolk bulged. Essentially this cleft is a magnified neurenteric canal, but since it does not pass through the primitive knot, it is an accessory neurenteric canal; the dorsal intestinal fistula is of this type.

The dorsal fistula may be compared with its ventral counterpart, the umbilical fisula or the patent form of Meckel's diverticulum. The diverticulum rarely remains patent; commonly it disappears completely, and between these two results is a wide variety of cysts and fibrous cords, indicating its partial degeneration. These forms may be present also with the dorsal fistula. The diverticulum runs through a fluid-filled cavity and at its complete disappearance leaves no trace. The dorsal fistula passes through all the median-line structures of the back, locally separating the two halves, and its former presence and later degeneration will be recorded in the more solid structures, vertebrae and spinal cord, in the form of a median defect of the vertebral bodies or a median spur arising from the body and penetrating the spinal canal; the spinal cord responds by the condition of diastematomyelia.

OBSERVATIONS ON THE HISTOGENESIS OF THE BRENNER TUMOR

Report of a Case Showing Ovarian and Endometrial Involvement

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A CASE of Brenner tumor of the ovary associated with exactly the same type of tumor in the endometrium is presented in this paper. Also, for the first time as far as is known, Brenner epithelium is reported occurring in the endometrium. The Brenner tumor of the ovary was detected accidentally after a panhysterectomy performed for "adenoacanthoma uteri," diagnosed on uterine curettings. When these uterine curettings were restudied and compared with the Brenner tumor of the ovary, the histologic findings were identical in both. As neither the ovarian nor the endometrial tumor showed any histological or cytological evidence of malignancy, the possibility of a primary cancer with metastases is excluded. Since the endometrium develops from the Müllerian system, and a tumor with the same cytologic features as the Brenner tumor of the ovary was found in it, this case, in a sense, supports R. Meyer's theory concerning the histogenesis of the Brenner tumor.

HISTORY AND PATHOLOGIC ANATOMY

The Brenner tumor is commonly described as a benign ovarian neoplasm characterized by islands of epithelial cells embedded in a dense fibrous stroma. It usually occurs as a small or moderate-sized, firm mass in an otherwise normal ovary or in the walls of a pseudomucinous cystadenoma. Microscopically, it shows islands of squamous epithelium of "transitional cell type." These cells are similar in size and shape throughout the island and show no evidence of keratinization. The epithelial islands are surrounded by a stroma of mature connective tissue. Frequently, small cavities appear in the centers of the epithelial islands, lined by columnar epithelium of mucinous type.[1] Three hundred nineteen cases of Brenner tumor had been reported in the literature up to 1946.[2] The first authentic description was that of a case reported by MacNaughton-Jones in 1898.[3] He called the tumor an "adenofibroma." A year later Orthmann[4] described a case, calling the

From The Department of Pathology and Clinical Pathology, New York Medical College, Flower and Fifth Avenue Hospitals.

1. Anderson, W. A. D.: Pathology, St. Louis, C. V. Mosby Company, 1948, p. 1120.

2. Giaccone, J. S.: Contributions from the Department of Gynecology of the City Hospital, New York, 1946, p. 16.

3. MacNaughton-Jones, M.: Tr. Obst. Soc. London **40**:154 and 213, 1898.

4. Orthmann, E. G.: Monatsschr. Geburtsh. u. Gynäk. **9**:771, 1899.

tumor "fibroma papillare superficiale carcinomatosum." Fothergill [5] reported two cases, and Glockner [6] one case, calling the growths solid carcinomata. Then, in 1907, Brenner [7] described three cases, noting that the epithelial nests in these ovarian tumors resemble the ovarian follicles in various stages of development and that the dense surrounding connective tissue could be the theca folliculi. Brenner called this tumor "oophoroma folliculare." In 1932 R. Meyer studied this ovarian tumor extensively and attached Brenner's name to it, giving him credit for the first thorough morphologic study. Meyer recognized that it was an entirely new type of ovarian tumor. He differentiated it from the granulosa cell tumor and described two forms of the Brenner tumor: one in which the tumor is composed of dense fibrous stroma containing islands of Brenner epithelium, and one in which fibrous nodules containing Brenner epithelium are found in the walls of pseudomucinous or serous cysts. Studying the Brenner tumors in detail, Danforth,[8] in 1942, and Arey,[9] in 1943, found another distinguishing cytologic characteristic of this tumor; they noted that the nucleus of the Brenner epithelium shows a grooving or folding, which is seen by high-power microscopic study and does not depend on the staining intensity of the nuclei. Arey noted that this cytologic feature was by no means constant. In some tumors it was present in certain regions and absent in others. Sometimes the nuclei that showed grooving were numerous, and sometimes they were scanty. Also, this type of nuclear grooving can be occasionally found in the nuclei of smooth muscle, connective tissue, and surface epithelium. As a rule, the Brenner tumor of the ovary is listed as a benign tumor, but Dubrauszky,[10] in 1949, reported a case of Brenner tumor of the ovary showing cytologic malignancy with hyperchromatic nuclei, giant cells, and beginning invasion.

CLINICAL FEATURES

The Brenner tumor occurs most frequently in postmenopausal women between the ages of 35 and 70. It has been reported occurring in a few younger individuals. The tumor is usually asymptomatic, but it may achieve a large size and can produce pressure symptoms. Schiffman [11] and Pendl [12] reported endometrial hyperplasia and an increased occurrence of postmenopausal bleeding in association with Brenner tumor. Taylor Jr. [13] stated that postmenopausal bleeding was found in cases of Brenner tumor associated with pseudomucinous cystoma. The cause of it, he believes, is a generalized hyperemia of the pelvis or substances elaborated in the ovarian cells in the walls of the cyst. But in most of the cases of Brenner tumor associated with postmenopausal bleeding extensive investigation has indicated that polyps, granulosa cell tumors, and uterine carcinomas were more acceptable causes of hemorrhage. The greater majority of the Brenner tumors reported, however, show no hormonal activity, and at present the tumor is regarded as nonfunctional.

5. Fothergill, W. E.: J. Obst. & Gynaec. Brit. Emp. **2**:15, 1902.
6. Glockner, A.: Arch. Gynäk. **75**:49, 1905.
7. Brenner, F.: Frankfurt. Ztschr. Path. **1**:150, 1907.
8. Danforth, D. N.: Am. J. Obst. & Gynec. **43**:984, 1942.
9. Arey, L. B.: Am. J. Obst. & Gynec. **45**:614, 1943.
10. Dubrauszky, V.: Geburtsh. u. Frauenh. **9**:473, 1949.
11. Schiffman, J.: Arch. Gynäk. **150**:159, 1932.
12. Pendl, O.: Wien. klin. Wchnschr. **26**:414, 1948.
13. Taylor, H. C., Jr.: Am. J. Obst. & Gynec. **36**:22, 1938.

HISTOGENESIS OF BRENNER TUMOR

There are several hypotheses referable to the histogenesis of the Brenner tumor of the ovary. The most acceptable are (1) Meyer's theory of Müllerian metaplasia, (2) Schiller's theory of embryonic urogenital epithelial inclusions, and a (3) teratomatous origin, supported by several authors.

The hypothesis proposed by R. Meyer [14] is apparently the one widely accepted. It suggests that the Brenner tumors arise from Walthard's nodules, which are undifferentiated cell rests of coelomic origin. These cells are similar to the undifferentiated cells lining the coelom, which gives rise to the Müllerian system. These undifferentiated cell aggregates can be found on the surfaces of ovaries, tubes, and uterine ligaments and also under the serosa of these organs. Walthard's nodules have been noted to have a tendency toward central necrosis and cyst formation, and they have been found to contain glycogen. The epithelial islands in the Brenner tumors also contain glycogen and show cyst formation. Meyer's belief was that as a result of metaplasia and differentiation of these totipotent cells, pseudomucinous adenomata with or without foci of undifferentiated cells (Brenner epithelium) may arise. Also solid Brenner tumors may develop from the undifferentiated rests, showing just fibrous connective tissue, undifferentiated cell islands, and small cysts. Even though Meyer described Walthard cell rests on the serosa of the tubes and the uterine ligaments, he mentioned that no Brenner tumors were found in these locations. Meyer believed that only some Brenner tumors arise from the Walthard cell rests. He stated that others are teratomatous in origin, and arise early in embryonal life. In 1934 Schiller [15] noted that Brenner epithelium resembled the transitional epithelium of the urinary tract and that the Brenner tumor resembled the picture of cystitis cystica. His theory was that the epithelial islands are inclusions of the embryonic urogenital epithelium. Schiller based his theory on a Brenner tumor in the hilum of the ovary in which he traced the transition of the rete ovarii epithelium to Brenner epithelium. He also demonstrated continuation between rete tubules and epoophoron tubules. Schiller did not claim that all Brenner tumors have to have the same origin. He believed that a part of them may arise from Walthard cell rests.

The teratomatous origin of the Brenner tumor has a few supporters. Plaut [16] found struma ovarii and a Brenner tumor in one ovary and a granulosa cell tumor in the other ovary. Brazilai [17] observed a great resemblance between the para-Malpighian cell aggregates (Walthard cell rests) and epidermoid cysts occurring in the ovaries. Dockerty and McCarty,[18] as well as Fox,[19] supported the theory of teratomatous origin.

REPORT OF A CASE

A 38-year-old white woman, single, was admitted to the Flower and Fifth Avenue Hospitals, New York, with a chief complaint of menorrhagia and irregularity of menstrual periods of six months' duration. Previously her menses occurred at 28-day intervals and lasted for four

14. Meyer, R.: Zentralbl. Gynäk. **56**:770, 1932.
15. Schiller, W.: Arch. Gynäk. **157**:65, 1934.
16. Plaut, A.: Arch. Gynäk. **153**:97, 1933.
17. Barzilai, G.: Atlas of Ovarian Tumors, New York, Grune & Stratton, Inc., 1943, pp. 145-161.
18. Dockerty, M. B., and MacCarty, W. C.: Am. J. Obst. & Gynec. **37**:703, 1939.
19. Fox, R. A.: Am. J. Path. **8**:223, 1942.

days. Lately menstruation occurred at 25-day intervals and lasted seven to eight days, with heavier flow throughout. No loss of weight was noted. Because of these menstrual changes, a diagnostic curettage was performed.

Microscopic study of the curettings showed sections of endometrium in which there was tumor tissue consisting of small tubular glands interspersed with sheets or nodules of epidermoid cells in the stromal areas. At the time, a diagnosis of well-differentiated adenoacanthoma of the endometrium was made. Because of this diagnosis, total hysterectomy and bilateral salpingo-oophorectomy were performed on the patient.

The pathological specimen consisted of a uterus and cervix measuring 13 by 11 by 9 cm. (Fig. 1). Both the tubes and the ovaries were attached. Section of the uterus showed a large, previously curetted endometrial cavity and a soft, pink, uniform myometrium. Grossly, there was no evidence of tumor involving the endometrium or invading the myometrium. The cervix showed minute cysts. The fallopian tubes were not significant. The left ovary measured 8 cm. in diameter and on section showed a cyst lined by a chocolate-colored membrane. In the wall of this cyst, replacing most of the ovary, there was a large, white nodule, 4 cm. in long diameter, which cut with considerable resistance. On section, this nodule showed numerous minute cysts.

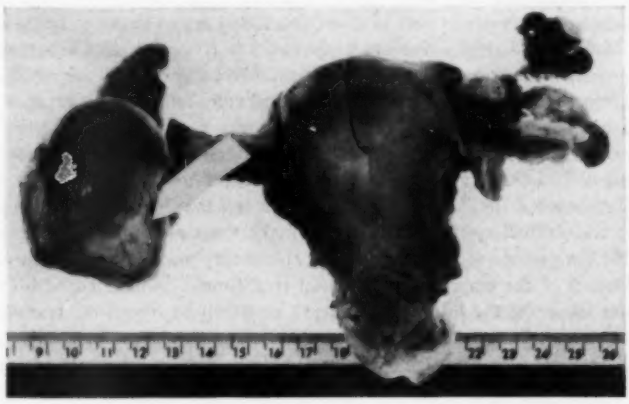

Fig. 1.—Uterus, both tubes, and ovaries. The left ovary shows a cross section of a large simple serous cyst with a Brenner tumor in its inferior wall.

The other ovary measured 3 cm. in diameter and on section revealed only one small lutein body in a uniform, moist stroma. Microscopic study of the uterus showed polypoid endometrial hyperplasia with focal calcification, acute hemorrhage, and inflammatory changes. There was no residual tumor in multiple sections examined.

Microscopic study of the solid portion of the left ovarian tumor showed multiple small and a few large cysts, some of which were lined by high, and some by low, columnar epithelium (Figs. 2 and 3). Other areas showed spheroid masses or nodules of epithelial cells. These cells had a very light eosinophilic cytoplasm and an oblong nucleus with low chromatin content. Distinct cell borders were not visible. The cells were uniform and of equal size. Some of these nodules had a cavity in the center, containing amorphous, deeply eosinophilic material and cellular debris. The epithelial cells lining these cavities were flattened. Among the nodules there were small cysts lined by one row of columnar, mucus-secreting epithelium. There were numerous transitions leading from the cysts lined by columnar epithelium into the above-described "epidermoid nodules." It actually appears as if the nodules represented an epidermoid metaplasia of the cyst epithelium. Between these epithelial structures there was a considerable amount of cellular fibrous connective tissue. The cyst grossly described as being lined by a chocolate-colored membrane was found to be a simple serous cyst with old hemorrhage showing hemo-

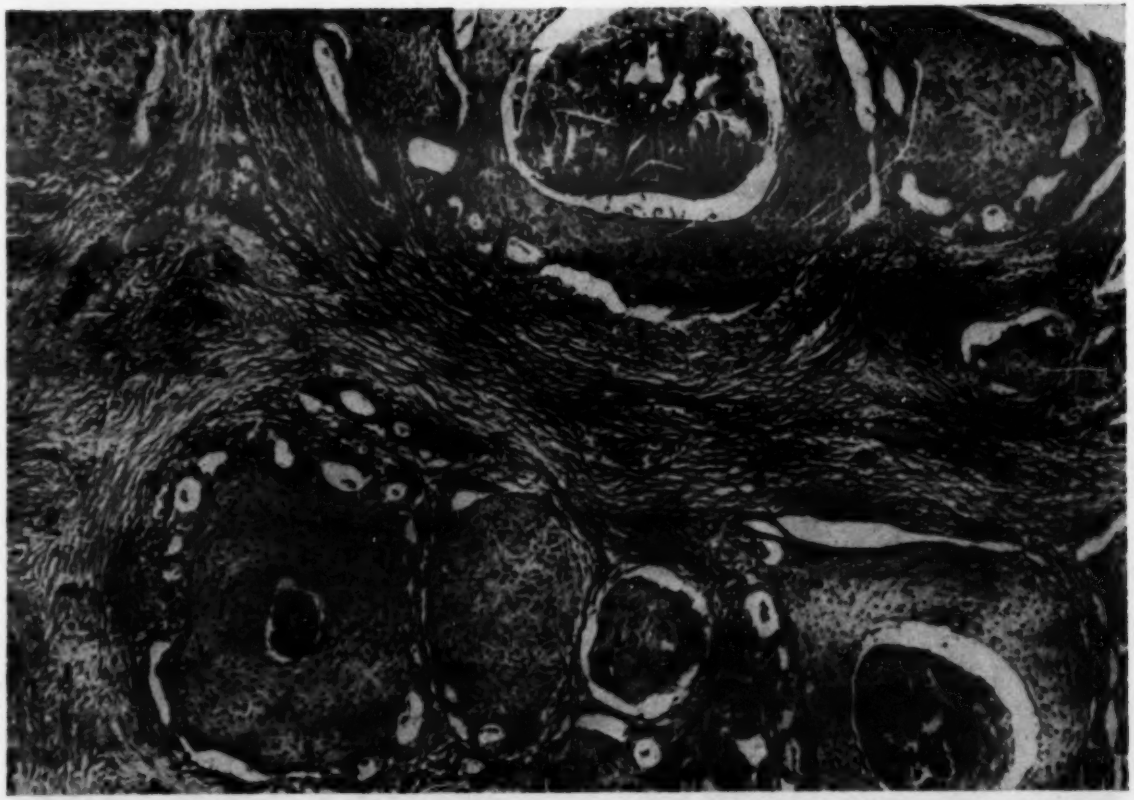

Fig. 2.—Brenner tumor of the ovary; × 100. Nests of Brenner epithelium with adjacent mucinous cysts are embedded in a dense fibrous stroma.

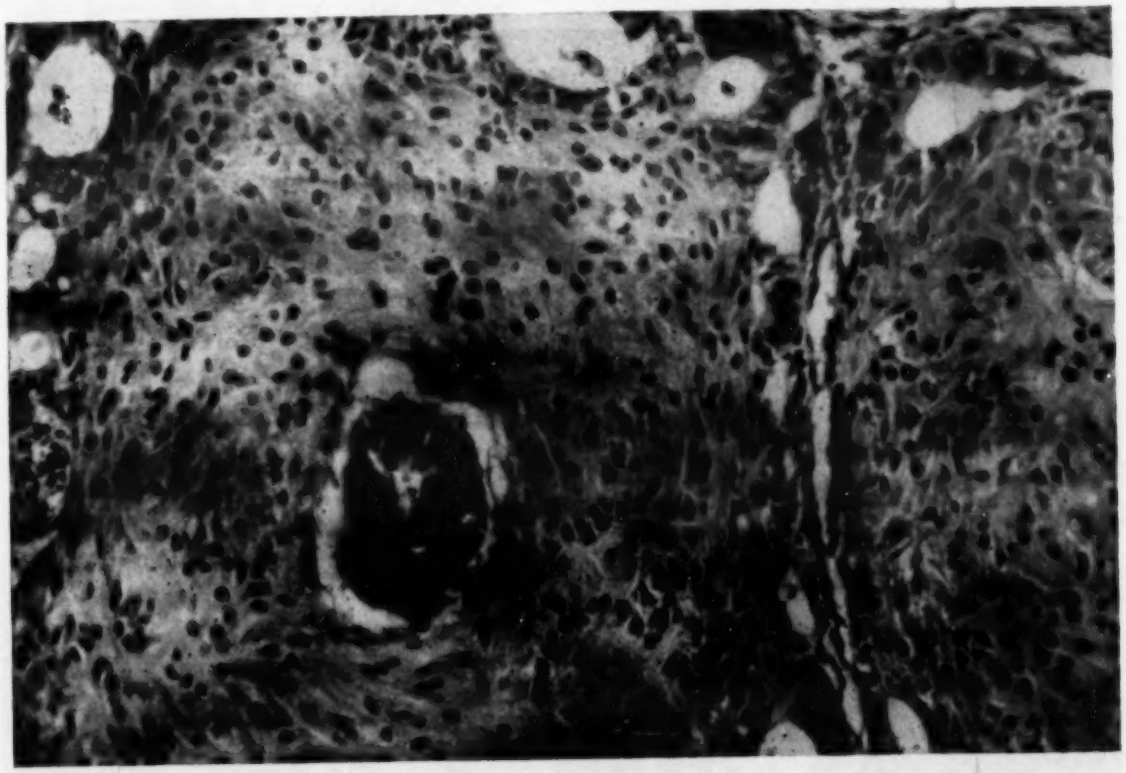

Fig. 3.—Brenner tumor of the ovary; × 430. A nest of Brenner epithelium with a necrotic center.

siderin-laden macrophages and giant cells. The remainder of the left ovary, as well as the right ovary, showed no significant changes. This ovarian tumor was diagnosed as a Brenner tumor in the wall of a serous cystoma.

The curettings, previously diagnosed as adenoacanthoma, were restudied. The major part of the curetted material was found to be identical with the above-described Brenner tumor (Figs. 4 and 5). There were nodules composed of elongated epidermoid cells, and many of them showed a small cavity lined by flattened epithelial cells. The nuclei of the epithelial cells showed no nuclear grooving or folding. In multiple areas the endometrial epithelium was seen merging with these epidermoid nodules. The connective tissue was that seen usually in the endometrium, except that it was present in greater amount and showed a tendency toward whorl formation encircling the epidermoid nodules. Several particles of the endometrium contained no Brenner tumor and showed an estrogenic-type endometrium with cyst formation. The endometrial glands per se showed no histologic criteria of cancer.

Multiple sections of the fallopian tubes were studied, and no tumor was found. This ruled out the possibility that the growth had extended from the ovary to the endometrium.

COMMENT

The exact similarity of the neoplastic changes in the endometrium and in the ovary, in this case, and the absence of infiltration or other evidence of cancer make it necessary to emphasize two points:

1. The endometrium is the seat of a Brenner tumor transformation, and the original diagnosis of adenoacanthoma was incorrect.
2. In this case, at least, it is proved that the Brenner tumor was derived from Müllerian elements.

Novak [20] noted that squamous cell metaplasia has always been seen in the endometrial adenocarcinomas of lesser malignancy (Broder's grading). This would explain why the adenoacanthomatous group gives relatively favorable results in therapy. We find that our endometrial squamous cell metaplasia (i. e., Brenner tumor) is very similar to that in a case described by Novak.[20] (See his Fig. 184.) That case was labeled as one of a "remarkable squamous metaplasia in a case of adenocarcinoma (grade I)." This observation suggests that some of the endometrial adenoacanthomas may be Brenner tumor transformations and that a restudy of cases of adenoacanthoma might reveal more tumors of the type herein reported.

The development of the Brenner tumor in the ovary and the endometrium can be explained on the following embryologic sequences. Kleine [21] mentioned that the so-called "germinal epithelium" covering the ovaries arises from the mesoderm which lines the coelom. The Müllerian ducts arise in the urogenital ridge and are also lined by coelomic mesoderm. The Müllerian epithelium differentiates into mucus-producing epithelium in the cervix, into squamous epithelium in the portio, into the endometrial epithelium, and into the tubal epithelium. As the surface epithelium of the ovaries originates from the same matrix as the Müllerian epithelium, it is possible that it maintains a similar ability for differentiation. The same ability of differentiation is used by Skinner and McDonald [22] to explain the development of adenoacanthoma in the endometrium. We believe that, referring

20. Novak, E.: Gynecological and Obstetrical Pathology, Philadelphia, W. B. Saunders Company, 1941, p. 202.

21. Kleine, H. O.: Ztschr. Geburtsh. u. Gynäk. **114**:125, 1936-37.

22. Skinner, I. C., and Mcdonald, J. R.: Am. J. Obst. & Gynec. **40**:258, 1940.

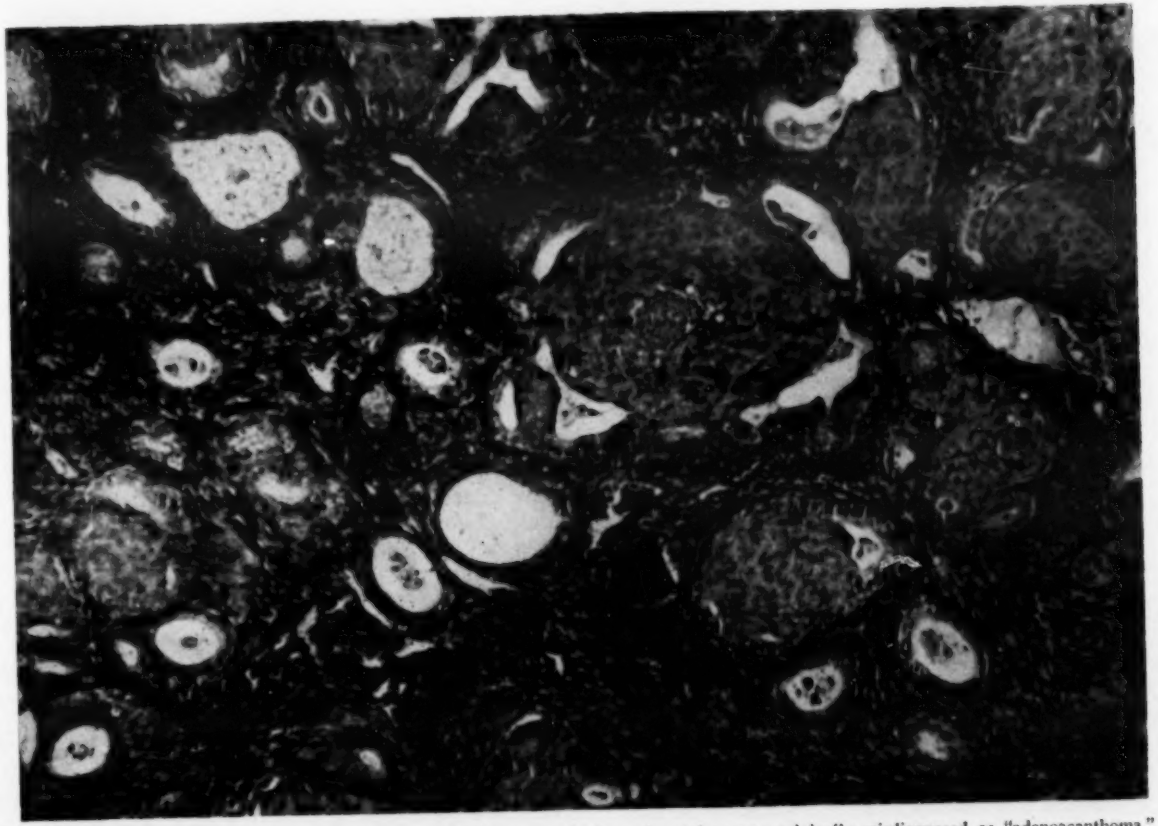

Fig. 4.—Uterine curetting; × 100. The Brenner epithelium seen in the endometrium was originally misdiagnosed as "adenoacanthoma."

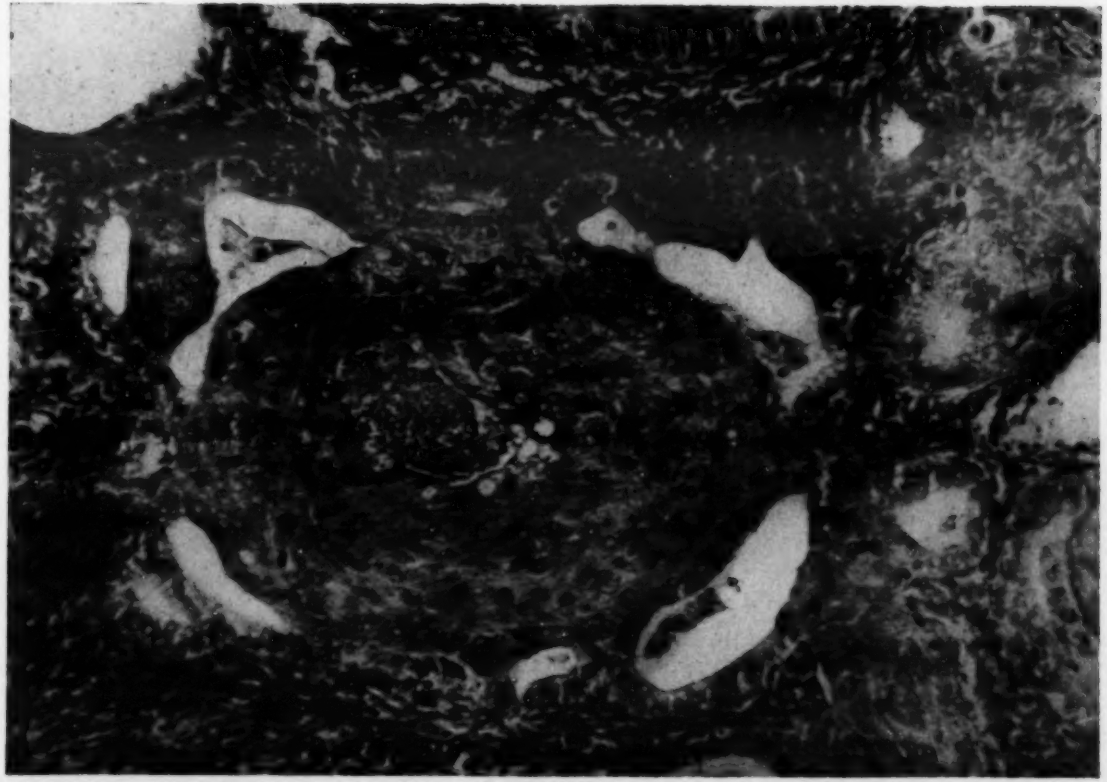

Fig. 5.—Uterine curetting; × 215. Brenner tumor of the endometrium showing Brenner epithelium with central necrosis and adjacent small mucoid-containing cysts.

to the above-mentioned embryologic development, we can regard the Brenner tumor as a form of squamous metaplasia of the Müllerian epithelium, and we can expect to find it also outside the ovary in any part of the Müllerian system.

SUMMARY AND CONCLUSIONS

A Brenner tumor of the ovary associated with Brenner epithelial transformation of the endometrium is reported. In this case the endometrial Brenner tumor was originally characterized as an adenoacanthoma. Such an error suggests that a restudy of cases of adenoacanthoma may reveal more Brenner transformations.

Meyer's theory that many Brenner tumors take origin from the Müllerian system thus receives support, since in this case identical Brenner epithelial transformation was found in otherwise normal endometrium and in an ovary containing a serous cyst.

OBSERVATIONS ON THE FORMATION OF SMALLPOX SCARS

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CLINICIANS familiar with the picture of smallpox agree that in this disease scarring is usually most pronounced in the face, a fact which is reiterated in many publications. Michelson and Ikeda,[1] for example, in a study of cases during the Minneapolis epidemic of 1925, reported that in three patients whom they were able to examine one year after recovery the smallpox scars were present predominantly on the face, although at the time of the illness the skin of the rest of the body had been affected to the same degree as the face. Ormsby and Montgomery,[2] in their current textbook on diseases of the skin, state that scars of smallpox "occur chiefly upon the face," thus giving rise to the pitting and "turning the babe into a changeling at which the mother shuddered, and making the eyes and cheeks of the betrothed maiden objects of horror to the lover."[3]

The uncomplicated smallpox lesion is essentially located within the epidermis; the corium suffers but little, and the lesion creates little opportunity for scar formation. Secondary bacterial invasion may, of course, cause ulceration with subsequent scarring, and it is well known that prevention of secondary infection by adequate nursing care and/or antibiotics prevents much of this scar formation. Stolte and Sas [4] recently demonstrated the value of chloramphenicol for this purpose.

It is still unexplained why the face should behave differently from other parts of the body and produce scars, i. e., pock marks. Textbooks on dermatology leave this question unanswered. Gans [5] refrained from any definite statement regarding this problem; he quoted Arzt in suggesting a possible difference in elastic fibers in the face. Michelson and Ikeda,[1] for want of better explanation, implicated the influence of sunlight without, however, going into any detail about the mechanism.

The object of the present study was to compare lesions of the face with those elsewhere on the body in order to determine the differences in the morphological appearances and to try to explain the peculiar tendency for pock marks to appear on the face.

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Present address of Dr. Bras: University College of the West Indies, Jamaica.

1. Michelson, H. E., and Ikeda, K.: Microscopic Changes in Variola, Arch. Dermat. & Syph. **15**:138, 1927.

2. Ormsby, O. S., and Montgomery, H.: Diseases of the Skin, Ed. 7, Philadelphia, Lea & Febiger, 1948, p. 507.

3. Macauly, cited by Haggard, H. W.: Devils, Drugs, and Doctors, New York, Harper & Brothers, 1946, p. 230.

4. Stolte, J. B., and Sas, J. J.: Chloramphenicol and A.C.T.H. in Smallpox, Lancet **2**: 715, 1951.

5. Gans, O.: Histologie der Hautkrankheiten, Berlin, Springer-Verlag, 1925.

MATERIALS AND METHODS

Material for this study was obtained from the autopsies of 177 cases examined during the epidemic of smallpox in 1949 in Batavia, Java. Complete details of the study were published in a monograph (Bras [6]); the present communication deals only with comparative findings in the skin of the face and of the thigh.

Blocks of skin from the face (taken from the region between the angle of the mouth and the mandibular angle) were compared with blocks from the skin of the anterior surface of the thigh. The tissues were routinely fixed in Helly's fluid,[7] and additional pieces were fixed occasionally in alcohol, formalin, Heidenhain's Susa mixture, or Peterson's fluid [8]; the blocks were embedded in paraffin. The sections were stained with the following stains: Harris' hematoxylin and eosin; Pranter's elastin stain occasionally combined with Unna's polychrome blue or nuclear fast red;

Fig. 1.—Confluent smallpox lesions in the face at the sixth day following eruption.

the McCallum-Goodpasture variant of the Gram stain, and connective tissue stains according to Wallart-Houette, Mallory, or Van Gieson.

OBSERVATIONS

Gross Findings.—The lesions of the face by the fifth day of illness were nearly always confluent and to a greater or less degree had assumed an eczematous appearance (Fig. 1), whereas elsewhere this phenomenon only very rarely occurred

6. Bras, G.: Variola: Een pathologisch-anatomische studie, Batavia, G. Kolff & Co., 1950.

7. Helly's fluid is a modification of Zenker's solution in which, instead of glacial acetic acid, formaldehyde solution (40%) is added in the concentration of 5%.

8. Peterson's fluid consists of equal amounts of aqueous mercuric chloride solution and Bouin's fluid.

(Fig. 2). Development of the lesion was faster in the face than on the rest of the body, i. e., confluent lesions, fully pustular in appearance, were present in the face after the fourth or the fifth day following eruption, and crust formation commenced on the eighth or the ninth day; in contrast, the rest of the body showed advanced pustular formation by the fourth or the fifth day, and crust formation lagged approximately four days behind that in the face.

Of the 177 patients considered in this study, 8 had died after the 15th day following eruption (2 of puerperal sepsis, 1 of encephalopathy, 4 of bronchitis and bronchopneumonia, 1 of generalized sepsis), and they presented the possibility of studying advanced stages of healing. Pock marks were unmistakably predominant in the face; areas of less pigmentation were usually the only remnant of the lesions elsewhere on the body.

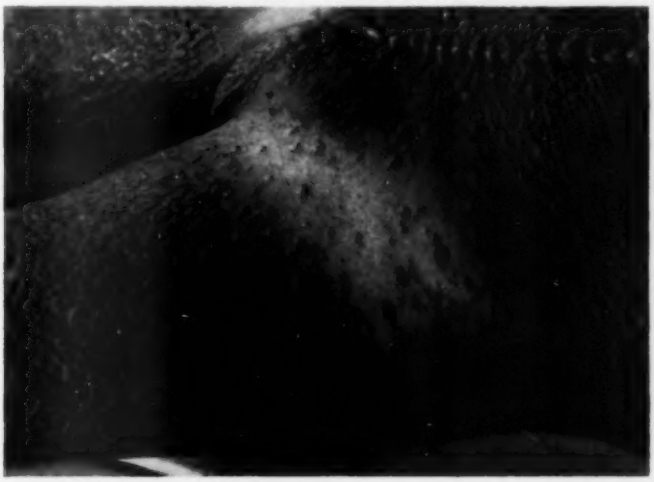

Fig. 2.—Discrete smallpox pustules in the thighs and lower abdominal regions at the ninth day following eruption; there is no confluence.

Histologic Aspects.—The formation and the structure of the vesicle in smallpox have been carefully studied by many. Lillie [9] in 1930 gave a complete review of the literature as known up till that time. Bras [6] gives full histological details as observed in his 177 cases.

For a clear understanding it may be repeated that in its early manifestation the smallpox lesion is essentially located within the epidermis. The cytoplasm of the spinal cells in the middle and upper parts of the dermis become swollen and more faintly staining. Intracytoplasmic vacuoles are formed, presenting an aspect to which Unna [10] applied the term "reticular colliquation." These cells eventually

9. Lillie, R. D.: Smallpox and Vaccinia, Arch. Path. **10**:241, 1930.

10. Unna, P. G.: Über den Sitz der Pocke in der Epidermis und die ersten Stadien des Pockenprozesses, Arch. Path. Anat. **69**:409, 1877; Histologischer Atlas zur Pathologie der Haut, Hamburg and Leipzig, L. Voss, 1900, No. 4.

lose their nuclei, the cell membranes rupture, and in this way a small vesicle is initiated. Through participation of many cells in the middle and upper layers of the stratum spinosum, a well-developed vesicle is usually present on the fourth or the fifth day following eruption. In the lower portion of the stratum spinosum and the stratum germinativum, i. e., under the vesicle, a different type of degeneration is visible—the so-called ballooning degeneration of Unna. (It was explained elsewhere [Bras [6]] that the name "ballooning degeneration" seems less appropriate in smallpox. This term was originally applied to a similar process taking place in varicella and herpes zoster, and in these two diseases true ballooning of cells is a common feature; however, this is not the case in smallpox.)

Fig. 3.—Vesicopustule from skin of the thigh at the sixth day following eruption. Note that the corium shows little involvement. Hematoxylin and eosin stain; × 43.

Very soon after this, the contents of the vesicles become cellular, and the pustule is formed. The granular and horny layers remain intact throughout the disease and form the roof of the vesicopustule. (In those lesions where the roof is torn, secondary bacterial invasion takes place, and the course of the disease is consequently altered.)

The morphological changes in the corium are very small indeed as compared with those in the epidermis. In the uncomplicated—i. e., not secondarily infected—smallpox lesion they do not exceed a localized endothelial proliferation in the subpapillary vessels, a stasis of mononuclear cells within these vessels, and a concomitant perivascular infiltration. Figure 3 shows a typical smallpox lesion of six

days' duration taken at autopsy from the skin of the thigh. It shows a vesicopustule, and it may be noted that practically all the pathological changes are situated within the epidermis; hardly any histopathological changes are visible in the corium.

However, severe changes of a distinctive character occur in the sebaceous glands and in those regions of the body where these glands are large and close together, i. e., the face; the subepidermal tissues are seriously affected as a result of this (Figs. 4, 5 and 6). The cells of these glands undergo a process of degeneration, showing at first cytoplasmic hyalinization with hyperchromatism of the nucleus, soon followed by detachment of the cell. Not long after this, karyorrhexis (Fig. 6) and cytolysis ensue. This degeneration usually occurs simultaneously in several

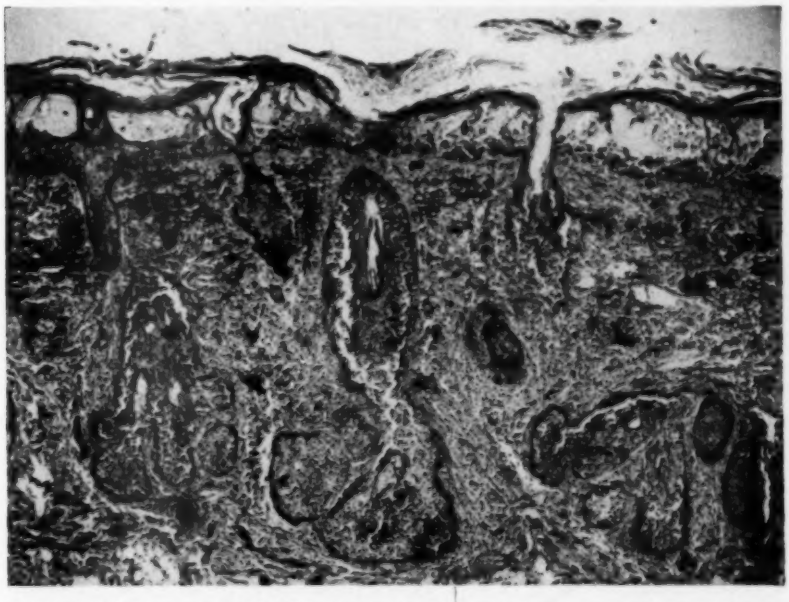

Fig. 4.—Vesicopustule in skin of the face, at the fifth day following eruption. Note the severe involvement of the subepidermal tissue through disintegration of the large sebaceous glands. Part of the middle one of the three glands is shown in greater detail in Figure 5. Hematoxylin and eosin stain; × 43.

foci of the same glands (Figs. 4 and 5) and spreads rapidly; eventually the complete gland becomes disrupted.

As a rule, bacteria were not demonstrated in hematoxylin-eosin- and Gram-stained tissues. This degeneration, then, evidently is not dependent upon secondary bacterial invasion but is an integral part of the smallpox lesion.

It will be evident that the destruction of the sebaceous glands causes a relatively major subepidermal defect in the face and a very minor one elsewhere, owing to the relative size and frequency of these structures at different sites of the body. a feature which is well illustrated in comparing Figure 3 with Figure 4.

Of the other dermal appendages, the sweat glands usually escape damage. In the hair follicles the cells of the juxtadermal portion display reticular and ballooning degeneration; the matrix is unaffected, and with passage of time the hair follicle regenerates completely.

The healing stage is characterized by drying up of the pustule. From all sides, newly formed epithelium grows under the lesion in a way which Buri [11] compared with the closing of an iris diaphragm. These regenerated cells undergo the normal

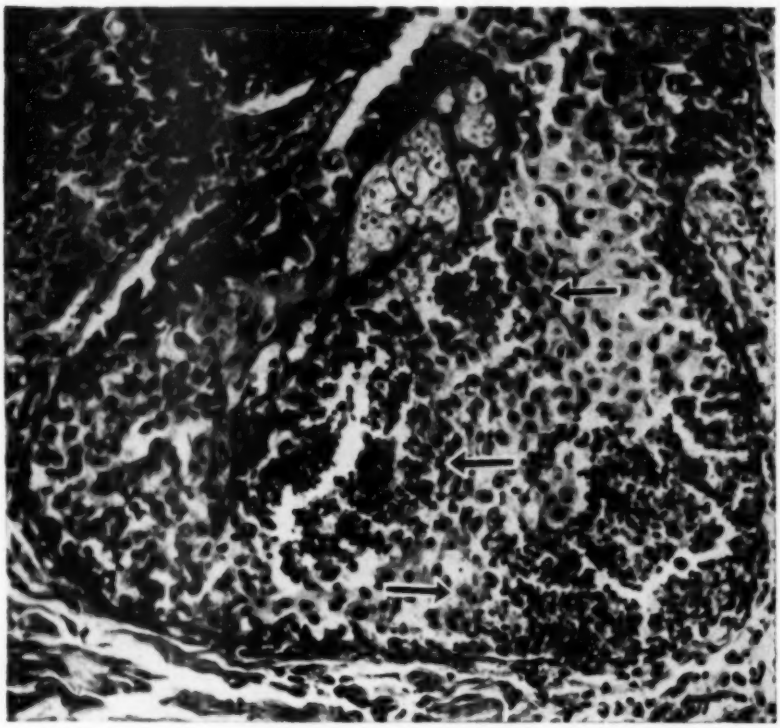

Fig. 5.—Gland depicted in Figure 4 shown in greater detail. Note foci of disintegration, indicated by arrows. Hematoxylin and eosin stain; × 260.

development into granular and horny layers, so that eventually the crust is trapped between two horny layers, the one originally present in the affected skin above and the newly formed one beneath. By the normal process of desquamation, the crust is shed. In areas where there is a thick stratum corneum, as in the palms and the soles, a longer time elapses before the crust reaches the surface.

11. Buri, T.: Die Anatomie der Variola- und Vaccinepustel, Monatsh. prakt. Dermat. **14**:20, 1892.

In the lesions not complicated by secondary bacterial invasions, very little reparative tissue reaction is needed to cope with the minor defects in the corium. No granulation tissue is produced, and the healed lesion leaves no scar; an area of flat rete pegs indicates the former site of the lesions. However, an exception must be made for the subepidermal defects caused by disintegrated sebaceous glands. In the face, these relatively large defects have to be filled with granulation tissue, and subsequent shrinking of this granulation tissue will cause cicatrization. Elsewhere no such large defects exist, and consequently no pock marks are produced. This explains the varying sequelae in the different locations.

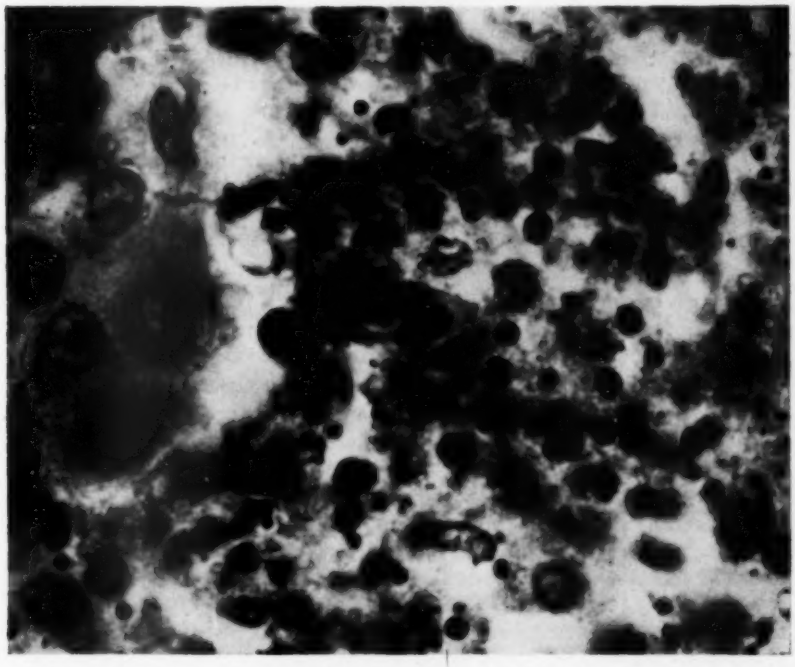

Fig. 6.—Destruction of sebaceous gland. Photograph of detail in Figure 5. In left half a few fairly normal looking cells are shown; in the center pyknosis and karyorrhexis are clearly visible. Hematoxylin and eosin stain; × 1540.

COMMENT

The suggestion by Michelson and Ikeda [1] that sunlight is the causative factor of scar formation cannot be accepted, since in tropical countries like Java many of the recovered patients go about stripped to the waist and yet show smallpox scars predominantly in the face.

Very little proof could be found for the suggestion that secondary infection is responsible for scarring; it is also difficult to understand why secondary infection, even when it does occur, should favor the face. In this series of cases, little evidence

of secondary bacterial growth could be seen, possibly because all patients had received antibiotic therapy.

Again, the material shows little difference in elastic tissue response as was suggested by Arzt.[12] The elastic fibers in the face we found not affected differently from the elastic fibers elsewhere in the body.

Bras [6] has demonstrated that the first appreciable skin lesion in smallpox is a cellular infiltration within and around the subpapillary vessels. The tributary regions supplied with blood through these vessels (i. e., the epidermis, the hair follicle distal from its neck, the sebaceous glands) suffer damages indicating a correlation between the vascular changes and subsequent histopathological aspects of the skin and appendages. In view of the fact that the skin of the face possesses an extremely well-developed subpapillary network of vessels (von Möllendorff [13]) it was thought possible that differences in the vascular supply might account for differences in the morphologic aspect of the lesion and/or its sequelae; detailed investigation, however, did not substantiate this.

A very significant difference between the face and the rest of the body, however, lies in the anatomical difference in size and distribution of sebaceous glands (compare Figures 3 and 4). These structures appear as large and closely set appendages in the face, and they undergo degeneration and necrosis as part of the histopathological process in variola. This necrosis also takes place in the sebaceous glands elsewhere in the body, but there, owing to their small size and sparse distribution, this defect is of only minor importance. In the face it is relatively considerable and causes a breach of continuity of the derma which is later filled with granulation tissue. This granulation tissue subsequently shrinks and causes scar formation.

Thus the pitting which is regularly seen in the face following smallpox infection is a consequence of degeneration of the closely set and relatively large sebaceous glands in this area of the body.

SUMMARY

As far as I am aware, no satisfactory explanation has been given to the problem of why scarring in smallpox is much more pronounced in the face than it is elsewhere on the skin. During the smallpox epidemic of 1949 in Batavia, Java, I performed over 175 autopsies on patients who died of this disease, and the formation of scars was one of the questions that was studied. For this purpose lesions occurring in the face were compared with those in the thigh, and the positive findings of this study are reported in this paper.

It was demonstrated that, of the dermal appendages, the sebaceous glands undergo a degeneration ultimately leading to necrosis, a fact which had been observed by previous investigators. However, it was not previously recognized that this causes a serious defect in the subepidermal tissue of the face because of the relatively larger size and greater number of the sebaceous glands in this area. The resultant scar formation causes the pock marks in the face to be appreciably different from those elsewhere on the body.

12. Arzt, L.: Zur Pathologie des elastischen Gewebes der Haut, Arch. Dermat. u. Syph **118**:470, 1913; cited by Gans.[5]

13. Handbuch der mikroskopischen Anatomie des Menschen, edited by W. von Möllendorff, Berlin, Springer-Verlag, 1927, Vol. 3, Pt. I.

DEGENERATION OF ELASTIC FIBERS IN GRANULOMATOUS GIANT CELL ARTERITIS (TEMPORAL ARTERITIS)

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AND
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CHARLOTTE, N. C.

THE SYNDROME of temporal arteritis has found steadily increasing attention since its initial description, by Horton, Magath, and Brown [1] in 1932. Today the condition has become a recognized distinct clinical entity. Its etiology is as yet obscure, and the histologic changes, although nearly pathognomonic, have not been recognized as specific. The concept of the disease is based largely upon the predominance of certain clinical and pathologic criteria, and, therefore, qualitatively is not definitive. Any additional observations which may shed light upon the distinctiveness of the condition, comparing it with similar ones, should be recorded. For this reason the following two cases are reported. From the discussion of our findings the reason for preferring the term "granulomatous giant cell arteritis" will become evident.

REPORT OF TWO CASES

CASE 1.—Three months prior to consulting us, a 65-year-old white woman began to have severe pain in the temporal area, first left, then right. Shortly, fatigue, malaise, and feverishness appeared. Our initial examination showed both temporal arteries to be tender, thickened, edematous, tortuous, and pulsatile. Low-grade fever, moderate arterial hypertension, moderate retinal arteriosclerosis, mild anemia, and a moderately increased erythrocyte sedimentation rate completed her clinical picture.

The excision of a segment of each temporal artery was followed by relief of pain. Gradual improvement of her general symptoms followed supportive symptomatic therapy. During a period of nine months there was no recurrence of arteritis.

Histologic Examination of Temporal Artery (S-1506-48).—Sections through various portions of the artery showed changes in all layers of the wall. The intima was greatly thickened by fibroblastic proliferation with much small round cell infiltration in its outer-most portion. Eosinophilic polymorphonuclear leucocytes were not conspicuous among the variety of infiltrating cells. Farther toward the lumen the thickened intima became less cellular, being mainly composed of collagenous tissue, the matrix of which had partially undergone mucinous degeneration. Greater cellularity was encountered in a narrow zone immediately next to the endothelium. In one of the sections there was a thrombus partially in process of organization. The media was preserved in its total thickness in only small portions, presenting very little inflammatory infiltration. Large segments of media were totally destroyed, being replaced by a very cellular inflammatory infiltrate of large mononuclear and small round cells and showing, in addition, a large number of multinucleated giant cells of the foreign-body type. These were crowded in portions of the media in which the inner elastic lamella was partially or totally destroyed. The majority of them were in immediate juxtaposition to the inner elastic

1. Horton, B. T.; Magath, T. B., and Brown, G. E.: An Undescribed Form of Arteritis of the Temporal Vessels, Proc. Staff Meet., Mayo Clin. **7**:700 (Dec. 7) 1932.

lamella (Fig. 1). In portions of the wall in which the inner elastic lamella was completely absent, it was difficult to decide whether they lay in the intimal or the medial zone of granulation tissue. The multinucleated giant cells were apparently concerned with the disintegration

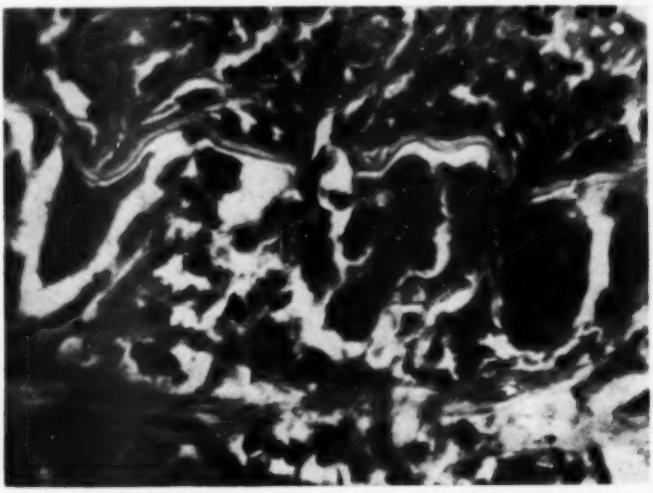

Fig. 1 (Case 1; S-1506-48).—Hematoxylin and eosin stain demonstrating relation between giant cells and the inner elastic lamella.

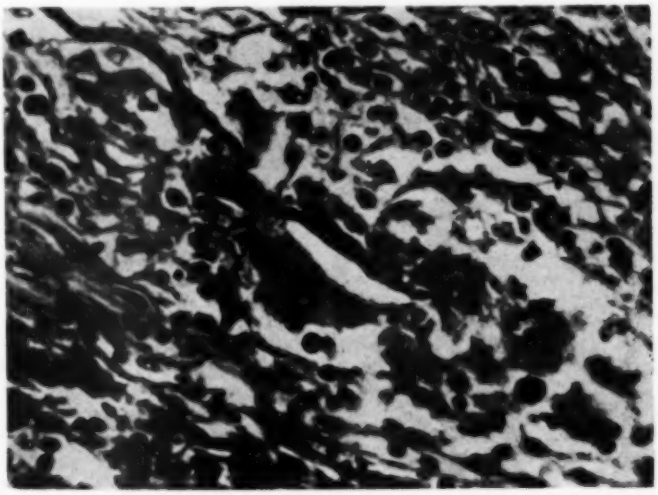

Fig. 2 (Case 1; S-1506-48).—Elastic tissue stain demonstrating giant cell "phagocytosis" of elastic membrane.

and removal of fragments of elastic fibers, since they were seen in immediate juxtaposition to the latter and also were found engulfing fragments of elastic tissue. The inner elastic lamella showed severe degenerative changes, manifest by swelling, fragmentation, blurring, and metachromasia (Fig. 2).

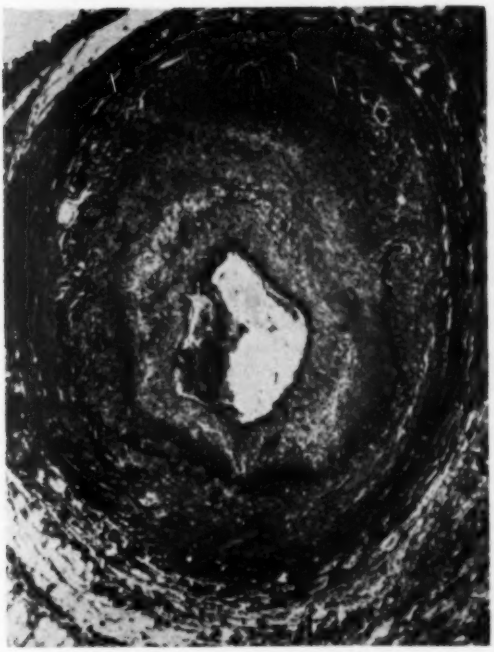

Fig. 3 (Case 1; S-1506-48).—Elastic tissue stain showing the essentially normal configuration of the outer contour of the artery, with central location of the lumen. The inner elastic membrane bulges outward in the upper half to almost merge with the outer membrane, the media being replaced by granulation tissue, the intima showing eccentric proliferation.

Fig. 4 (Case 1; S-1506-48).—Elastic tissue stain showing an "intramural aneurysm" with partial destruction of the inner elastic lamella and giant cell accumulation in this particular area.

A low-power survey of hematoxylin-eosin-stained sections showed the inflammatory infiltration and the aggregation of giant cells situated seemingly in the media. The muscle bundles had disappeared and were replaced by the above-mentioned granulation tissue. Elastic tissue stains, however, showed that, although the outer configuration of the artery on cross section was rather well preserved, the thinning of the media and the granulation tissue replacement of the media were taking place in areas in which the inner elastic lamella bulged outward and in which the "aneurysm" was filled with thickened intima (Fig. 3). In this manner the inner lining of the lumen and the outer circumference of the artery were in concentric arrangement, whereas the inner elastic lamella showed outward bulging in some places and had completely disappeared in others. It was for this reason that much of the granulation tissue and the giant cells, upon superficial examination, seemed to be situated in the media, although in reality the rows of giant cells and the adjacent dense small-round-cell infiltration were in juxtaposition to the displaced inner elastic lamella in portions of the wall in which the media had been partially destroyed. This situation may be designated as "intramural" aneurysm of the internal elastic membrane (Fig. 4).

There was much irregular inflammatory infiltration of the adventitial tissue, including some, though not all, of the vasa vasorum. Some inflammation was seen in perivascular lymph spaces of adjacent smaller vessels which did not seem to be in direct continuity with the larger artery. There was only an irregular and insignificant inflammation of medium-sized veins in the connective and fat tissue.

CASE 2.[2]—Two months before we saw her, a 67-year-old white woman began to have severe left occipital pain. The pain extended to both temporal areas and became intolerable. Slight fever, malaise, and weakness appeared.

The temporal vessels bilaterally were acutely tender, thickened, nodular, and tortuous. Tenderness was noted also over the carotid arteries. Slight fever, mild arterial hypertension, and mild anemia were present. The electrocardiogram showed left bundle-branch block.

The excision of a segment of each temporal artery was followed by about 75% pain relief. Follow-up has not been accomplished.

Histologic Examination of Temporal Artery (S-2961-50).—Most of the sections through this artery were taken in longitudinal fashion. A description of the histologic details appears redundant, since the features were essentially the same as those described in the previous case.

GENERAL COMMENTS

Since the original publication by Horton, Mageth, and Brown,[1] in 1932, numerous additional cases have been reported.[3] An excellent review was published by Harrison[4a] in 1948. There has been objection to the term "temporal arteritis,"

2. Dr. J. E. Anthony, of Kings Moutain, N. C., gave us permission to report this case.

3. (*a*) Horton, B. T., and Magath, T. B.: Arteritis of the Temporal Vessels: Report of 7 Cases, Proc. Staff Meet., Mayo Clin. **12**:548 (Sept.) 1937. (*b*) Bowers, J. M.: Arteritis of Temporal Vessels: Report of a Case, Arch. Int. Med. **66**:384 (Aug.) 1940. (*c*) Scott, T., and Maxwell, E. S.: Temporal Arteritis: A Case Report, Internat. Clin. N. S. **42**:220 (June) 1941. (*d*) Shannon, E. W., and Solomon, J.: Bilateral Temporal Arteritis with Complete Loss of Vision, J. A. M. A. **127**:647 (March 17) 1945. (*e*) Schaeffer, C. L., and Sanders, C. E.: Temporal Arteritis, Am. Heart J. **24**:410 (Sept.) 1942. (*f*) Gilmour, J. R.: Giant-Cell Chronic Arteritis, J. Path. & Bact. **53**:263 (Sept.) 1941. (*g*) Chasnoff, J., and Vorzimer, J. J.: Temporal Arteritis: A Local Manifestation of a Systemic Disease, Ann. Int. Med. **20**:327 (Feb.) 1944. (*h*) Horton, B. T.; Magath, T. B., and Brown, G. E.: Arteritis of the Temporal Vessels: A Previously Undescribed Form, Arch. Int. Med. **53**:400 (March) 1934. (*i*) Haining, R. B., and Kimball, T. S.: Polyarteritis Nodosa, Am. J. Path. **10**:349 (May) 1934. (*j*) Krahulik, L.; Rosenthal, M., and Loughlin, E. H.: Periarteritis Nodosa (Necrotizing Panarteritis) in Childhood with Meningeal Involvement: Report of a Case with Pathologic Findings, Am. J. M. Sc. **190**:308 (Sept.) 1935.

4. (*a*) Harrison, C. V.: Giant-Cell or Temporal Arteritis: Review, J. Clin. Path. **1**:197 (Aug.) 1948. (*b*) Cohan, H., and Harrison, C. V.: Temporal Arteritis: Report of 3 Cases, ibid. **1**:212 (Aug.) 1948.

and some prefer to use the term "cranial arteritis." Several authors suggest that the lesion is not restricted to the cranial arteries but is a systemic vascular disease. There is evidence to support this view.[5] Ocular symptoms, including pain, glaucoma, and varying degrees of visual loss, have been observed in 30 to 50% of the cases of temporal arteritis. Indeed, in some cases retinal vessels are reported to show pathologic changes [6] similar to those seen in the temporal arteries. Further evidence of the systemic nature of the disease is the frequent occurrence of general symptoms, such as fever, malaise associated with muscle aches, generalized soreness, and the like.

The description of the histologic changes in arteries removed from patients who show characteristic local and constitutional signs and symptoms is conspicuously uniform. The granulomatous lesions of the artery are located mainly in the media and the intima, associated with intimal proliferation, organizing thrombosis, and segmental destruction of the inner elastic lamella. The latter is characterized by the presence of numerous multinucleated giant cells. Although the pattern of this composite picture is quite distinctive, it cannot be denied that any single criterion may also occur in other vascular diseases. A discussion of the differential diagnosis would be repetitious, since the salient features have been presented elsewhere in detail (Kilbourne and Wolff,[5a] Crosby and Wadsworth,[7] Cooke and associates,[8] and others).

It should be stated, however, that the differences in the pattern of tissue reaction in temporal arteritis outweigh by far the resemblances to other forms of nonsuppurative arteritis and, therefore, call for separate classification. The finding of degenerated elastic fragments in giant cells, in our opinion, adds greatly to the specificity of the morphologic picture and deserves detailed consideration.

Lesions of Elastic Fibers

Temporal Arteritis.—The observation to which we refer is not original. Gilmour [5f] described minute fragments of elastic fibers in, or in contact with, multinucleated giant cells but expressed doubt that the giant cells were essentially connected with destruction of elastic tissue. Sproul and Hawthorne [9] spoke of giant cells containing bits of elastic fibers, and Crosby and Wadsworth [7] presented a photomicrograph of a foreign-body giant cell reacting to degenerated elastica. Plaut [10] referred to multinucleated giant cells as having been excited by fragments

5. (*a*) Kilbourne, E. D., and Wolff, H. G.: Cranial Arteritis: A Critical Evaluation of the Syndrome of "Temporal Arteritis" with a Report of a Case, Ann. Int. Med. **24**:1 (Jan.) 1946. (*b*) Gordon, L. Z., and Thurber, D. C.: Temporal Arteritis: Report of a Case and a Comparison with Respect to Periarteritis Nodosa, Arch. Path. **42**:402 (Oct.) 1946. (*c*) Jennings, G. H.: Arteritis of the Temporal Vessels, Lancet **1**:424 (Feb. 19) 1938. Gilmour.[5f] Harrison.[4a] Cohan and Harrison.[5b]

6. Dick, G. F., and Freeman, G.: Temporal Arteritis, J. A. M. A. **114**:645 (Feb. 24) 1940. Dantex, D. A.: Temporal Arteritis, ibid. **131**:1265 (Aug. 17) 1946. Schaeffer and Sanders.[8e]

7. Crosby, R. C., and Wadsworth, R. C.: Temporal Arteritis: Review of Literature and Report of 5 Additional Cases, Arch. Int. Med. **81**:431 (April) 1948.

8. Cooke, W. T.; Cloake, P. C. P.; Govan, A. D. T., and Colbeck, J. C.: Temporal Arteritis; Generalized Vascular Disease, Quart. J. Med. **15**:47 (Jan.) 1946.

9. Sproul, E. E., and Hawthorne, J. J.: Chronic Diffuse Mesaortitis: Report of 2 Cases of Unusual Type, Am. J. Path. **13**:311 (March) 1937.

10. Plaut, A., in discussion on Sproul, E. E.: Case of "Temporal Arteritis," New York J. Med. **42**:345 (Feb. 15) 1942.

of elastic fibers, and Harrison [4a] and Cooke and associates [8] emphasized the close relationship between giant cells and destroyed elastic fibers. The cases of Sproul and Hawthorne [9] and those of Gilmour [8f] did not involve the temporal arteries and the identity of the condition they observed and so-called temporal arteritis, for this and other reasons, has been questioned (Crosby and Wadsworth [7]).

A survey of the literature would lead one to believe that the appearance of multinucleated giant cells can be traced to irritation by fragments of elastic fibers in only a relatively small number of accepted cases of temporal arteritis. Harrison,[4a] in his survey, stated, "Some giant cells lie in relation to fragments of elastic tissue, but others occur in the thickened intima in situations away from demonstrable elastica."

We have not been able to find in case reports in the literature a detailed histologic description or photomicrographs which showed conclusively the presence of giant cells in portions of the wall unrelated to the elastic tissue. The report of Harrison's own three cases [4b] describes and illustrates the giant cells as occurring only in the media. In addition to the sections studied in our two cases, one of us had the opportunity of reviewing sections in several hitherto unreported cases of temporal arteritis which revealed features similar to those described in our cases. Fragments of elastic tissue were found within and in immediate contact with multinucleated giant cells (Figs. 5 and 6).

There are several reasons for the relationship between giant cells and elastic fibers being either misinterpreted or overlooked. In the first place, the giant cells are conspicuously massed immediately exterior to, or within, the plane of the inner elastic lamella and are found in greatest numbers in segments of the arterial wall in which the elastic membrane is absent. The impression may be gained that they are located in the media. In reality, however, they are often situated in that portion of the wall where the media is totally destroyed and the configuration of the remaining inner elastic lamella indicates an intramural aneurysmatic bulge, with preservation of the outer and inner contour by organizing granulation tissue. The position of giant cells in such areas indeed suggests that they were originally external and in juxtaposition to the inner elastic membrane which is now destroyed. The rather delicate elastic fibers in the media may disappear within the granuloma, leaving no demonstrable fragments within giant cells.

Secondly, giant cell "phagocytosis" [11] of elastic fibers may not be seen in every section. In some of our cases it was observed in only approximately two-thirds of numerous sections. Unless careful search is made, a negative finding cannot be evaluated.

Finally, it should be pointed out that an adequate specific stain is of importance. We have found resorcin-fuchsin elastic tissue stain superior to others and have applied one of Papanicolaou's staining methods as a counterstain, which renders the cytoplasm of giant cells clear and translucent. Also, in the process of degeneration, presumably assisted by the activity of giant cells, the fragments of elastic fibers become invisible. Various phases of this gradual disappearance can be observed.

The changes in the elastic lamellae can be described as swelling and fragmentation. In some portions their affinity to any of the specific stains is greater; elsewhere they may become less intensely stained, the colors varying from blue to brownish

11. The term "phagocytosis" is used loosely here and is not intended to convey the conventional connotation.

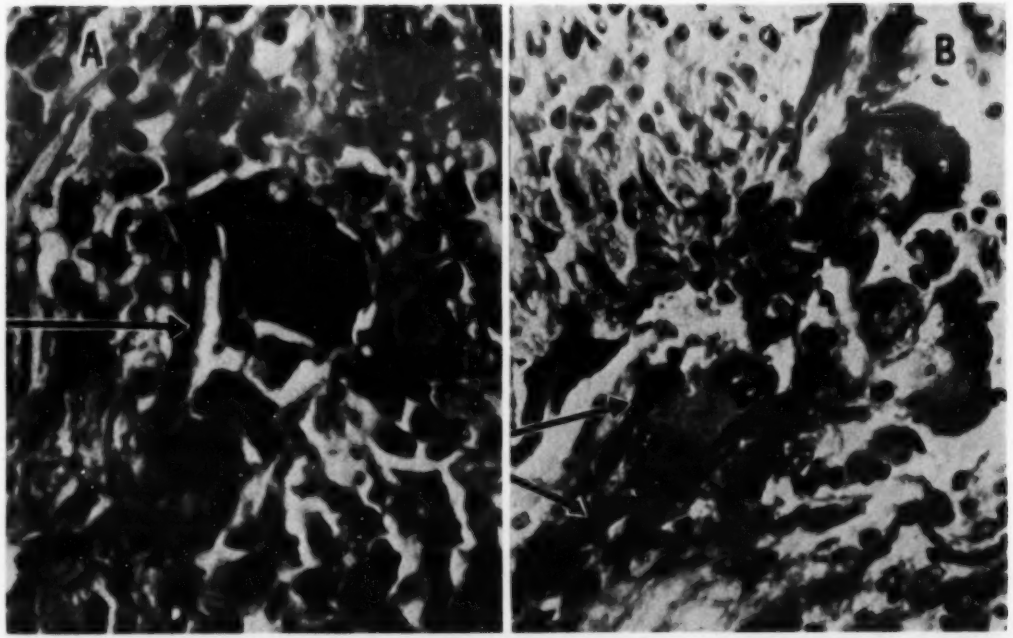

Fig. 5.—Examples of giant cell "phagocytosis" of elastic membrane. Arrows indicate elastic fibers. *A* (Case 2; S-2961-51), hematoxylin and eosin stain. *B* (43-3150), elastic tissue stain.

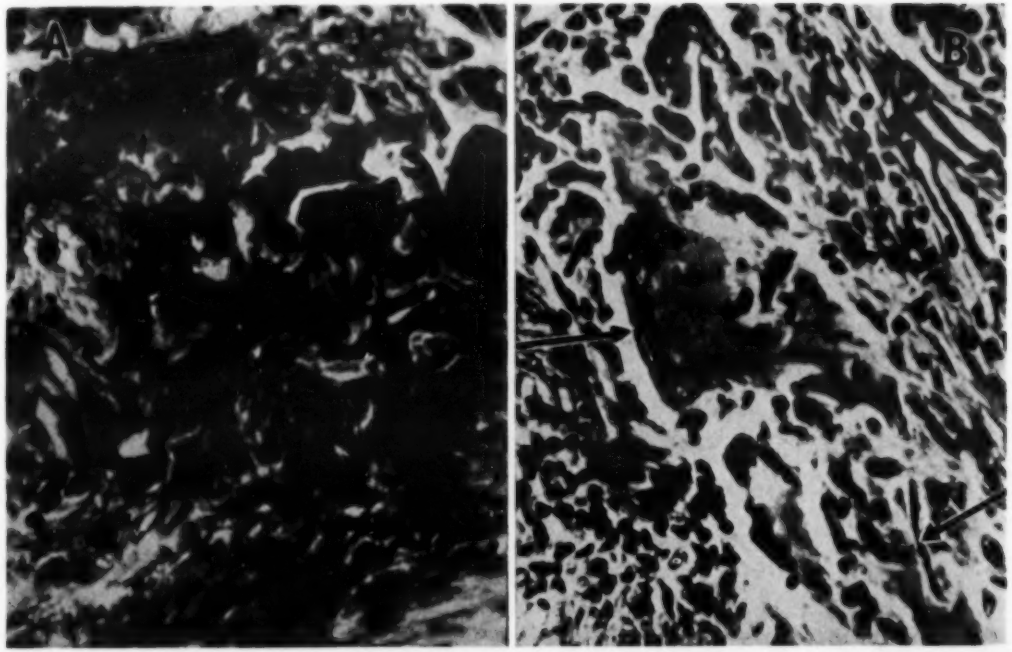

Fig. 6 (40-5187; 40-5516).—Elastic tissue stains demonstrating further examples of giant cell "phagocytosis" of elastic membrane. In *B* arrows indicate elastic fibers.

red. We have not observed axial crystalloid structures, described by Haas,[12] but one can readily see the increased refraction. In the degenerated portions the fibers often lose their undulation and give the impression of being rigid. The sharply pointed ends are seen to "pierce" the giant cells.

Senility.—There is a conspicuous absence of giant cell reaction in the changes of elastic elements taking place in the various phases and forms of arteriosclerosis. In the temporal arteries, however, lesions have been observed which are believed to be distinct and different from those seen in arteriosclerosis elsewhere. In connection with dilatation and tortuosity, Schmidt [13] described defects of the inner elastic lamella associated with corresponding thickening of the intima and occasional calcification. He interpreted these defects as "tears" due to a peculiar local disposi-

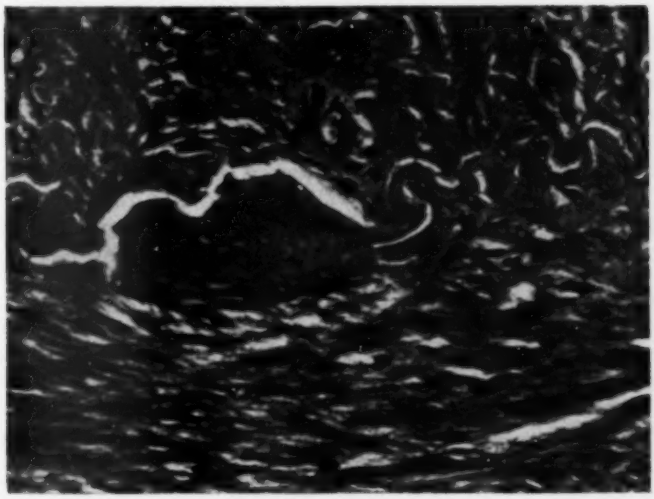

Fig. 7 (A-1384).—Temporal artery with perielastic hyalinosis; hematoxylin and eosin stain.

tion of temporal arteries. He did not mention granulomatous reaction to such tears of elastic membrane.

We have examined temporal arteries in a series of 50 elderly persons in order to compare common senile changes with those seen in temporal arteritis. For the most part the lesions were those generally classified as atherosclerosis or arteriosclerosis.

Aside from the well known alterations of elastic elements in arteriosclerosis, we have noticed a lesion which seems to have escaped previous attention. We were unable to find a description of this distinctive change. In several instances the hematoxylin-eosin-stained sections revealed discrete hyaline-like homogeneous nodular

12. Haas, G. M.: Elastic Tissue: III. Relations Between the Structures of the Aging Aorta and the Properties of the Isolated Aortic Elastic Tissue, Arch. Path. **35**:29 (Jan.) 1943.

13. Schmidt, M. B.: Über die Schlängelung der Arteria temporalis, Zentralbl. allg. Path. u. path. Anat. **30**:49, 1919.

swellings of the internal elastic lamella. An analysis with various stains revealed that the material was deposited in the perielastic connective tissue and that the elastic lamella proper frequently passed unaltered through the masses. Treated with Schiff's reagent according to the method of McManus,[14] the material seemed to contain carbohydrate. In contrast to a statement by Menten and Carpenter,[15] the elastic membrane proper remained completely unstained (Fig. 7).

Close examination of temporal arteries revealed that the changes in the matrix of the connective tissue in immediate juxtaposition to the inner elastic lamella often appear either mucoid in type or as an ill-defined narrow zone of homogenization, which becomes conspicuous if it appears in its discrete nodular form. It was seen only in the older age group (average, 68 years) but not always associated with other common manifestations of arteriosclerosis. The elastic membrane proper, as indicated above, may appear entirely intact, but may also be broken up, thickened, rigid, and calcified. Although the various degenerative changes of the elastic membrane may occur in conjunction with perielastic hyalinosis, the two processes may be found independent of each other. Perielastic hyalinosis does not seem to be confined to the temporal arteries. We have found occasional changes of this type in other small arteries.

Senile lesions in the temporal arteries, whether taking place in the elastic membrane proper, in the perielastic tissue, or in other layers of the wall, even if associated with a segmental defect, did not show giant cell reaction in our control series.

PATHOGENESIS

Our concept of the pathogenesis of this granulomatous arteritis is based upon these observations in our two cases, the study of sections of other unpublished cases,[16] and on inference from histologic descriptions of reported cases. It is apparent that such conclusions require further investigation and reexamination of previously reported cases in the light of these observations.

In general, it may be stated that "phagocytosis" of fragments of elastic fibers is not commonly observed. In fact, it is difficult to find specific information on this subject. The only direct reference we have been able to find was that to obliterating bronchiolitis in which giant cells were observed containing inclusions which could be demonstrated only with elastic tissue stain.[17] This process is described as suggestive of phagocytosis. Jores,[18] in his chapter on degenerative changes of elastic fibers, made no mention of giant cell reaction.

It is our opinion that so-called temporal arteritis is a specific nonsuppurative granulomatous arteritis, characterized by a peculiar destruction of elastic fibers and

14. McManus, J. F. A.: The Histological Demonstration of Mucin After Periodic Acid, Nature **158**:202 (Aug. 10) 1946.

15. Menten, M. L., and Carpenter, A-M.: The Histochemical Distribution of Glycogen, A. M. A. Arch. Path. **51**:486 (May) 1951.

16. Dr. Tracy Mallory let us study the sections of six cases from the files of the Department of Pathology of Massachusetts General Hospital.

17. Hart, C., and Mayer, E., in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer-Verlag, 1928, Vol. 3, Pt. 1, p. 481.

18. Jores, L., in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer-Verlag, 1928, Vol. 2, p. 640.

resulting in giant cell reaction. In this manner it distinguishes itself from other degenerative and inflammatory lesions of arteries in which elastic fibers may disintegrate.

It is true that giant cells are occasionally, though infrequently, found in other forms of arteritis, but there is no evidence that they are related to destruction of elastic fibers in those conditions. This is obvious in specific types of arteritis, such as tuberculosis, syphilis, and rheumatic granulomatosis, in which the giant cells are part and parcel of the specific granulomata anywhere in the wall of the vessel. In the rare instances of periarteritis nodosa in which giant cells are found they bear no consistent relationship to any layer of the arterial wall and in some instances are described as being located in and around the adventitia (Haining and Kimball [21]; Krahulik, Rosenthal, and Loughlin [31]). Buerger's endarteritis resembles temporal arteritis more than any other form of arteritis. The occurrence of giant cells in this disease is estimated as frequent by some authors (for instance, Crosby and Wadsworth [7]) and as rare by others (Allen, Barker, and Hines [19]). The preservation, however, of the inner elastic lamella is one of the outstanding features of Buerger's endarteritis, in which it contrasts with temporal arteritis. Moreover, giant cells directly related to elastic fibers are not described in Buerger's endarteritis; in fact, the giant cells occur most frequently in organized thrombi.

We postulate that giant cell arteritis may actually have its origin in a peculiar lesion of the elastic membrane, the etiology of which is not determined. This concept is in keeping with the fact that the major portion of the granuloma is found external to the inner elastic membrane, since the lymphatic drainage from the intima flows in the direction toward the adventitia. Also, the loss of inner elastic membrane exposes the media to a localized increase of pressure which results in an incomplete intramural aneurysm, such as those seen in one of our cases, in one of the cases of Crosby and Wadsworth,[7] and in a dissecting aneurysm of the aorta in the case of McMillan.[20] The necrosis of musculature and connective tissue matrix of the media, which is often described, may be secondary to the stress and strain in this area which is no longer protected by the inner elastic lamella. Thickening of intima, thrombosis, and inflammation of vasa vasorum are most likely secondary phenomena.

Cooke and associates [8] thought that the process begins in the vasa vasorum and that the changes in the media and the inner elastic lamella result from ischemia due to obliteration of nutrient vessels. This explanation is difficult to accept. In the first place, it is doubtful that the inner elastic lamella derives its nutrition solely from these vessels. Secondly, Cooke and associates assumed that the process spreads in axial direction, presumably, therefore, beyond the region of supply by the initially obstructed vasa vasorum. Furthermore, the involvement of the vasa vasorum by inflammatory infiltration or hyalinization is irregular in distribution and severity, and not all of the vessels are involved. There is no evidence that the changes in these vessels are older than those in the wall of the main artery. Inflammation of vasa vasorum is common in arteriosclerosis, in which the primary change is known to occur in the intima. We agree with Crosby and Wadsworth [7] that it is not possible at this time to arrive at any conclusion in regard to the role the vasa vasorum may play in the pathogenesis of granulomatous arteritis.

19. Allen, E. V.; Barker, N. W., and Hines, E. A.: Peripheral Vascular Diseases, Philadelphia, W. B. Saunders Company, 1947.

20. McMillan, G. C.: Diffuse Granulomatous Aortitis with Giant Cells, Associated with Partial Rupture and Dissection of the Aorta, Arch. Path. 49:63 (Jan.) 1950.

Most authors seem to agree that the process begins in the media and that in medium-sized arteries the main lesion is most commonly found next to the inner elastic lamella. Our own concept of the histogenesis, as outlined above, is in keeping with these observations.

Distribution

We are as yet not familiar with the extent to which granulomatous arteritis may involve the vascular tree. Clinically, the disease is dominated by local signs and symptoms directly referable to the involvement of certain segments of the arterial system with predominance of that of the temporal arteries and its branches. A generalized lesion of the arterial system, however, is indicated by the constitutional signs and symptoms, supported by the observation of characteristic changes in various visceral and parietal arteries.

A number of cases involving the aorta and its larger branches have been excluded by Crosby and Wadsworth [7] for the reasons that the age group, the symptoms, and the pathologic aspects were different from those of typical temporal arteritis. Revision of these cases, however, on the basis of the criteria to which we have referred may lead to reevaluation. The clinical symptomatology is vague and inconclusive if vessels other than temporal arteries are involved. Therefore, whether the cases of Sproul and Hawthorne [9] and Gilmour [2f] belong to the group of cases of so-called temporal arteritis can be decided only on morphologic grounds. The granulomatous giant cell reaction to fragments of elastic bodies seems to favor including these cases in the group under discussion.

Conclusions

Temporal or cranial arteritis has been looked upon as a distinct clinical syndrome. However, the underlying pathologic process, though characteristic in its pattern, has not been recognized in the past as pathognomonic. Our observations and our interpretation of case reports in the literature have led us to conclude that the structural changes characterized by giant cell digestion of elastic fibers are sufficiently distinctive to justify the acceptance of a specific clinicopathologic entity. Since the lesion is not confined to the temporal arteries, and most likely not to cranial arteries, but is probably of systemic distribution, we suggest that the condition be classified according to its most outstanding pathogenetic feature, namely, the giant cell reaction to disintegrating elastic fibers. The term "granulomatous giant cell arteritis" introduced by J. R. Gilmour [2f] appears appropriate.

Summary

Two cases of giant cell arteritis involving temporal arteries are reported.

Changes of elastic fibers in temporal arteries were studied. It was found that (*a*) "temporal arteritis" is characterized by a granulomatous giant cell reaction to disintegrating elastic fibers and that (*b*) senile lesions of temporal arteries, in addition to common arteriosclerotic changes, may consist of defects of the internal elastic lamella and a peculiar perielastic hyalinosis.

On the basis of pathogenetic characteristics, the term "granulomatous giant cell arteritis" is preferred rather than the regional designation of "temporal" or "cranial arteritis."

The condition is recognized as a specific clinicopathologic entity.

NEPHROTOXIC GLOBULIN NEPHRITIS

II. Relation of Severity to Adrenal Size

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WHEN NEPHROTOXIC serum nephritis is produced in the rat by administration of rabbit anti-rat-kidney serum the manifestations are highly variable in their nature and in their severity. It was suggested in the first paper of this series [1] that an analysis of the causes of variability might clarify the knowledge of the pathogenetic mechanism, and the course after a single intravenous injection was described in detail.

Previous investigators, notably Smadel and Farr [2] and Heymann and Lund,[3] performed experiments in which the schedule and the route of administration were inconstant, male and female, prepubescent and adult animals were mixed, and the nephrotoxic serum used was of inconstant and unmeasured potency. In the present series of investigations the effect of these variables, and some others, is under evaluation. We have found in our work that when the resultant nephritis was classified by arbitrary standards the grade of nephritis was related to adrenal size and the excretion of neutral reducing lipid, regardless of the variable factors mentioned above. Some of these results have been published in preliminary form [4]; it is the purpose of this paper to describe in detail the grades of nephritis arbitrarily established and their relation to adrenal size.

It is well to reiterate clearly that nephrotoxic globulin nephritis, while it has many features in common with human glomerular nephritis and "lipoid" nephrosis, also has features of clear distinction. The gross hematuria that is a cardinal sign of initial glomerular nephritis in the human subject is almost never seen in the rat. On histologic examination of the kidney the signs of inflammation are usually

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Dr. Harry Goldblatt examined and interpreted the tissue sections. Mrs. Ruth T. Lackey contributed technical assistance.

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1. Lippman, R. W.; Marti, H. U., and Campbell, D. H.: A. M. A. Arch. Path. **53**:1, 1952.

2. Smadel, J. E.: J. Exper. Med. **64**:921, 1936. Smadel, J. E., and Farr, L. E.: ibid. **65**:527, 1937. Smadel, J. E.: ibid. **65**:541, 1937.

3. Heymann, W., and Lund, H. Z.: Science **108**:448, 1948; Pediatrics **7**:691, 1951.

4. Lippman, R. W., and Marti, H. U.: Proc. Nat. Acad. Sc. **37**:447, 1951.

minimal and the deposits of glycoprotein, so vividly stained in basement membranes of human kidneys from patients with glomerular nephritis and lipoid nephrosis, are far less prominent in the rat. Nevertheless, a study of controllable influences upon the pathogenesis of this particular experimental nephritis may be expected to yield information about the patterns of renal tissue responses.

METHODS AND MATERIALS

Nephrotoxic Globulin (NTG) and Control Globulin (GC).—Purified nephrotoxic gamma globulin was prepared [5] from rabbit anti-rat-kidney serum in the manner previously described.[1] One lot (3/17/50) had a total protein concentration of 32.5 mg./ml., and a second lot (4/12/51) had a total protein concentration of 25.5 mg./ml. In order to produce lesions of varied intensity, the amount of NTG given was varied from 0.15 ml. to 0.6 ml., given in a single intravenous injection, in multiple subcutaneous injections, or by both routes.

The control globulin preparations were made by similar procedures from the serum of normal, unimmunized rabbits. One preparation, with a total protein concentration of 53.0 mg./ml., was administered by multiple subcutaneous injections, on the same schedule as the comparable NTG injections, on Monday, Wednesday, and Friday, for four weeks. The second preparation, with a total protein concentration of 17.0 mg./ml., was administered in a single intravenous injection of 0.6 ml.

Experimental Procedure.—For these experiments, 116 male rats of the Slonaker-Addis strain were used. Each animal weighed 150 gm., with little variation. Six groups of 12 to 15 animals each were given NTG by the various routes of administration and dosage schedules mentioned. Of these, 10 died during the experiments, most as a result of the initial shock of intravenous NTG administration, and these have not been considered in the groupings to be described.

Two control groups of 12 animals each were used, one group which received GC by multiple subcutaneous injections, as described, and one group which received a single intravenous injection of GC. The animals were kept for four weeks after the first globulin injection.

Before each experiment started a four-hour urine collection was made to determine the rate of excretion of urinary protein. During the experiment all the animals were fed the stock diet, which contains 17% protein, and received water ad libitum, except during periods of urine collection. At these times the animals received only 10% dextrose solution with 0.4% sodium chloride and 0.5% betaplexin.[6]

At the end of the fourth week, an eight-hour urine collection was made, and the animals were then killed by exsanguination from the abdominal aorta during light ether anesthesia. The following data were obtained: wet weights of kidneys, heart, adrenal glands, testes, and liver. The presence of ascites was noted, and the quantity of fluid was measured. Clotted blood and an oxalated sample were obtained for hematocrit and erythrocyte sedimentation rate (Wintrobe) determination. The total drawn blood volume was measured. The eight-hour urine volume was measured, and the serum was examined for visible lipemia which, when present, was graded from 1 to 4+. In each of the individual urine specimens the protein content was determined by the method of Stafford and Shevky.[6] In some groups blood pressure determinations were made, before the urine collection, by the tail-microphone method of Friedman and Freed.[7]

Tissues of kidney, adrenal glands, and liver were fixed for microscopic examination in neutral 10% formalin in 0.85% sodium chloride solution. Paraffin sections were stained with hematoxylin and eosin, by the Mallory technique to demonstrate connective tissue elements, and by the periodic acid-Schiff technique. Frozen sections of adrenal glands, kidneys, and livers were stained for fat with Sudan Black B or Sudan III.

Individual animals were then assigned to groups according to the arbitrary classification system to be described below. Pools were made from the urine and the serum by combination of aliquot portions from animals with the same group classification in each experiment. The

5. Dr. Dan H. Campbell and Jay Banovitz, of the California Institute of Technology, prepared the nephrotoxic globulin and the control globulin.

6. Shevky, M. C., and Stafford, D. D.: Arch. Int. Med. **32**:222, 1923.

7. Friedman, M., and Freed, S. C.: Proc. Soc. Exper. Biol. & Med. **70**:670, 1949.

neutral reducing lipid excretion was determined in some groups by a modification of the method of Heard and Sobel.[8]

The endogenous urea clearance was calculated in all groups from serum and urine urea determinations, performed by the method of Kibrick and Skupp.[9] The endogenous creatinine clearance was calculated from serum and urine creatinine determinations performed by the method of Bonsnes and Taussky.[10] Serum and urine chloride determinations were performed by the method of Schales and Schales.[11] In addition to the individual protein determinations previously mentioned, the pooled urine specimens were analyzed by the biuret method of Kingsley,[12] and this method was also used for determination of the total serum protein concentration. The total serum cholesterol concentration was measured by a slight modification of the method of Bloor, Pelkan, and Allen.[13] The total serum lipid concentration was measured by the method of Bloor.[14]

Classification of the Lesions.—In order to evaluate the variable lesions produced by the administration of nephrotoxic globulin in the rat it was decided to classify them according to arbitrary standards, using characteristics that could readily and rapidly be determined. For this purpose five characteristics were chosen: change in kidney weight from the value predicted for a normal rat of the same size,[15] change in heart weight from the predicted value, rate of urinary protein excretion, presence and degree of ascites, and presence and degree of visible

TABLE 1.—*Standards for Classification of Nephrotoxic Globulin Nephritis*

Rating	Kidney Wt. Increase, %	Heart Wt. Increase, %	Proteinuria, Last Day, Mg./100 Gm./24 Hr.	Visible Lipemia	Ascites
0	Less than 0	Less than 0	0-10	0	0
1	0-10	0-10	11-25	+	+ (just noticeable)
2	11-50	11-15	26-50	++	++ (up to 5 ml.)
3	51-100	16-25	51-250	+++	+++ (5-15 ml.)
4	101 and over	26 and over	251 and over	++++	++++ (over 15 ml.)

Each animal was rated for each factor in the table. Then the total score for each rat was used to assign a group: Group N (0-1), Group A (2-5), Group B (6-10), Group C (11-15), Group D (16-20).

lipemia. The arbitrary standards of rating were chosen in order to divide the experienced range of variation into three segments, with an additional low rating for the normal range and an additional high rating for unusually high values. The classification system is given in Table 1. After classification, as previously mentioned, specimens of serum and urine were pooled from rats of the same group in each experiment.

RESULTS AND COMMENT

General Observations.—The general behavior and appearance of animals that receive nephrotoxic globulin (NTG) and control globulin (GC) by intravenous injection have previously been described in detail.[1] The same observations and

8. These values have been reported previously [4]; the determinations were performed by Dr. Harry Sobel and Herschel Greenfeld.

9. Kibrick, A. C., and Skupp, S.: Proc. Soc. Exper. Biol. & Med. **73**:432, 1950.

10. Bonsnes, R. W., and Taussky, H. H.: J. Biol. Chem. **158**:581, 1945.

11. Schales, O., and Schales, S. S.: J. Biol. Chem. **140**:879, 1941. Asper, S. P., Jr.; Schales, O., and Schales, S. S.: ibid. **168**:779, 1947.

12. Kingsley, G. R.: J. Biol. Chem. **131**:197, 1939.

13. Bloor, W. R.; Pelkan, K. F., and Allen, D. M.: J. Biol. Chem. **52**:191, 1922.

14. Bloor, W. R.: J. Biol. Chem. **77**:53, 1928.

15. Addis, T., and Gray, H.: Growth **14**:49 and 81, 1950.

comments pertain to this group of experiments. However, in this series some of the animals died within a short time after the injection, when they had been given a large amount of NTG. No obvious cause of death was seen at autopsy. The hemolysis discussed in the previous report was evidenced by slight jaundice of the subcutaneous tissues and mucosal surfaces but was not very noticeable. In addition, some of the animals showed signs of pulmonary edema, with frothy, blood-tinged sputum. The animals that received subcutaneous injections of NTG appeared to be perfectly normal until the third and fourth weeks, when a lag from the normal growth rate became evident.

Effect upon Body Growth.—It was previously reported that animals to which nephrotoxic globulin had been administered showed a lag in the body growth when compared with the animals given control globulin.[1] It can be seen from Table 2 that in the present experiments the growth lag is roughly related to the severity of the nephritis and of a magnitude similar to that previously observed. Appetite and food consumption were not measured but were not grossly impaired in Groups

TABLE 2.—*Relation of Body and Organ Weights to Severity of Nephrotoxic Globulin Nephritis*

Group	Animals	Body Weight, Gm.	Kidney Wt., % of Predicted	Heart Wt., % of Predicted	Adrenal Wt., % of Predicted	Liver Wt., % of Predicted	Testes Wt., % of Predicted
Control	23	214	+ 3	+ 4	+ 9	+ 5	+23
NTG-N	12	203	− 6	− 3	+15	+ 1	+26
NTG-A	20	208	+ 3	+ 5	+22	+12	+15
NTG-B	26	198	+ 28	+10	+30	+32	+21
NTG-C	29	191	+ 85	+19	+70	+57	+ 1
NTG-D*	2	180	+136	+22	+62	+59	+ 5

*Because only two animals fell in Group D, the results in this group were not considered significant unless consistent with the results in Group C.

N, A, and B. In Groups C and D food consumption may have been reduced, since the animals were manifestly unwell and were azotemic.

Organ Weight.—Since the increase in relative heart weight was a factor in grouping, it was to be expected that relative heart weight would increase in the order of Groups N to D, and this was found to be the case, with a maximum increase of 22% in Group D. This increase in relative cardiac size can be attributed to work hypertrophy, associated with the increase in systemic blood pressure to be described. Rather[16] has shown in rats of the same strain that there is a close correlation between cardiac size and blood pressure, and this can also be seen from the present data in Figure 1.

Adrenal gland weight showed a slight but significant relative increase over the control value even in Group N, with a progressive increase to the maximum of about 70% in Groups C and D.

The relative liver weight in Group N was not significantly different from the control value. However, a striking and progressive increase in relative liver weight was observed in Groups A to D, with a maximum increase of nearly 60% in Groups C and D. The increase in liver weight might be attributed to edema of the liver, deposition of fat as a result of the gross disorder of lipid metabolism to be described,

16. Rather, L. J.: Am. J. Physiol. **159**:153, 1949.

or deposition of glycogen as a result of increased adrenal cortex activity. Studies of tissue sections, including special stains to demonstrate lipid and glycogen deposition, do not support these possibilities. Synthesis of serum proteins by the liver is an endergonic reaction and requires the performance of work. The nephritic animals lose massive amounts of serum protein in the urine, in Groups C and D a mean amount each day which is approximately three times the total circulating protein for a normal rat of the same size.[17] Therefore, it might be expected that

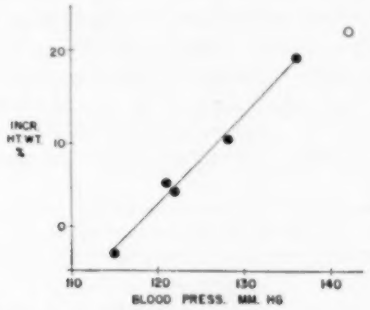

Fig. 1.—Relation of the increase in relative cardiac size to mean blood pressure in the rat with nephrotoxic globulin nephritis. The curve was not weighted by the value from Group D (open circle) because of the small number of observations.

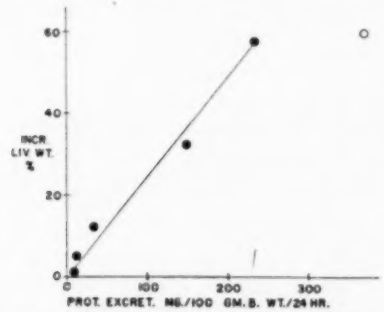

Fig. 2.—Relation of the increment of relative liver weight to protein excretion in the rat with nephrotoxic globulin nephritis. The curve was not weighted by the value from Group D (open circle) because of the small number of observations.

the relative increase in liver size would result principally from true work hypertrophy. If this hypothesis were so, the increment in liver size should be directly related to the increment in work performed. The latter quantity should be a function of total protein excretion, which must be equivalent to the increment in protein synthesis in order to maintain a dynamic equilibrium in the total amount of circulating protein. This relation is demonstrated in Figure 2.

17. Lippman, R. W.: Proc. Soc. Exper. Biol. & Med. **67**:193, 1948.

The relative weight of the testes was more variable than the relative weight of other organs, and the relative weight in Groups N, A, and B could not be considered to vary significantly from the relative weight in the control globulin group. In Groups C and D there was a significant diminution in relative testicular weight.[18] Antopol [19] showed that cortisone administration inhibits testicular development and spermatogenesis in the mouse testis. The diminution of testicular size observed in our experiments may well have resulted from the increased secretion of adrenal hormones, some with androgenic activity, associated with adrenal hypertrophy and increased excretion of neutral reducing lipid, to be mentioned.

Proteinuria.—Animals in the control globulin group did not have significantly increased proteinuria in view of the known values for protein excretion determined in normal male rats of our colony and a closely related colony.[20] Since the degree of proteinuria was a factor in the grouping, it was expected that proteinuria would increase in the order of groups, and this was found to be the case, with no increase

TABLE 3.—*Relation of Proteinuria, Blood Pressure, Ascites, and Certain Other Observations to Nephrotoxic Globulin Nephritis*

Group	Protein Excretion, Mg./100 Gm. B. Wt./24 Hr.	Total Serum Protein, Gm./100 Cc.	Eryth. Sed. Rate, Mm./Hr.	Drawn Blood Vol. Ml./100 Gm. B. Wt.	Ascites, Ml.	Blood Pressure, Mm. Hg
Control	12	5.40	6	3.6	0	122
NTG-N	9	5.39	9	3.2	0	115
NTG-A	35	5.13	4	3.4	0	121
NTG-B	150	4.33	11	3.2	0	128
NTG-C	241	3.60	23	3.4	1.0	136
NTG-D	372	3.26	..	3.0	1.0	142

of protein excretion from the control value in Group N and a maximum protein excretion rate of 372 mg./100 gm. of body weight/24 hr. in Group D (Table 3).

Total Serum Protein Concentration.—There was no significant difference between the total serum protein concentration found in Group N and the value in the control group. In the remaining groups there was a progressive decline in concentration to 3.26% in Group D.

Drawn Blood Volume.—It has previously been shown that the drawn blood volume obtained under constant conditions is roughly proportional to the actual blood volume.[21] No significant differences of drawn blood volume were noted in these experiments. From this observation we can infer that no grossly obvious change occurred in blood volume.

18. In all instances where the significance of data was not obvious on inspection, significance was tested by the method of R. Fisher (Statistical Methods for Research Workers, New York, Hafner Publishing Company, Inc., 1950). $P < 0.01$ was considered to indicate a significant difference.

19. Antopol, W.: Proc. Soc. Exper. Biol. & Med. **73**:262, 1950.

20. Addis, T.; Marmorston, J.; Goodman, H. C.; Sellers, A. L., and Smith, M.: Proc. Soc. Exper. Biol. & Med. **74**:43, 1950.

21. Lippman, R. W.: Proc. Soc. Exper. Biol. & Med. **66**:188, 1947.

Erythrocyte Sedimentation Rate.—The erythrocyte sedimentation rate observed in Groups N and A did not differ significantly from the control group value. In Groups B and C there was a progressive rise in the sedimentation rate.

Ascites.—Ascites of mild degree was observed only in Groups C and D. Edema was not considered in the grouping system because of the difficulty involved in precise measurement. However, gross edema was not observed in any instance at the kill, or at any time during the experimental period. Most animals in Groups C and D also had small bilateral pleural effusions, usually less than 1 ml.

Blood Pressure.—Blood pressure measurements were made in only half of the animals in each group. A statistically significant rise of blood pressure over the control value of 122 was observed only in group C, with a value of 136. This group also was found to be azotemic.

Urine Volume.—The urine volume was quite variable within each group and no significant difference from the control value was noted in Groups N and A. In

TABLE 4.—*Relation of Various Renal Function Measurements to Severity of Nephrotoxic Globulin Nephritis*

Group	Urine Vol., Ml./ 100 Gm. B. Wt./ 24 Hr.	Serum Creat. Conc., Mg./ 100 Cc.	Endogen. Creatinine Clearance		Serum Urea Conc., Mg./ 100 Cc.	Endogen. Urea Clearance Ml./Min./ Gm. K. Wt.	Serum Chloride Conc., Mg./ 100 Cc. (as NaCl)	Urinary Chloride Excret., Mg./ 100 Gm. B. Wt./ 24 Hr. (as NaCl)
			Ml./Min./ Gm. Kidney Wt.	Ml./Min./ 100 Gm. B. Wt.				
Control	37.5	0.69	0.670	0.457	31.1	0.723	668	129
NTG-N	49.2	0.66	0.569	0.384	30.0	0.574	695	135
NTG-A	42.0	0.68	0.520	0.449	29.4	0.587	667	132
NTG-B	25.5	0.71	0.552	0.486	34.4	0.486	645	134
NTG-C	17.6	0.68	0.317	0.292	53.7	0.235	678	87
NTG-D	8.2	0.81	0.155	0.254	89.8	0.103	742	141

Group B the urine volume was significantly diminished, and in Group C the diminution of urine volume was consistent and striking, with a value of 17.6 ml./100 gm. body weight/24 hr. compared with a control value of 37.5 ml. (Table 4). In Group D the value was 8.2 ml.

Serum Creatinine Concentration and Endogenous Creatinine Clearance.—The previous paper of this series [1] noted changes in the endogenous creatinine clearance with the passage of time after a single intravenous injection of nephrotoxic globulin if the clearance was calculated in terms of the kidney weight measured at autopsy. In the present experiments no significant differences were found in the serum creatinine concentration or in the endogenous creatinine clearance when the latter was calculated in terms of body weight, except for a significant diminution of clearance in Group D. When calculated in terms of measured kidney weight, however, the endogenous creatinine clearance declined significantly to a minimum value in Group D, which was less than one-fourth of the control value. These observations led to a conclusion similar to the one which was previously given: The renal enlargement observed after nephrotoxic globulin administration resulted principally from an increase in nonfunctional renal mass (with respect to creatinine excretion), with a diminished excretion capacity per unit of renal weight, although

the whole organ remained capable of meeting the creatinine excretory load, except in Group D, as indicated by failure of the serum creatinine concentration to rise significantly.

Serum Urea Concentration and Endogenous Urea Clearance.—The serum urea concentration did not change significantly from the control value in Groups N and A. In Group B there was a slight rise, of possible significance, while in Groups C and D distinct azotemia was present, with a serum urea concentration nearly triple the control value in the latter group. In addition, there was a progressive and striking fall in the endogenous urea clearance when this was calculated in terms of body weight or the kidney weight measured at autopsy.

Serum Concentration and Urinary Excretion of Chloride.—With the exception of Group D, in which the results were of doubtful significance because of the small number of observations, there were no significant changes in the serum chloride concentration. Group C showed the only significant change in chloride excretion,

TABLE 5.—*Relation of Serum Lipid and Neutral Reducing Lipid Excretion to the Severity of Nephrotoxic Globulin Nephritis*

Group	Animals	Lipemia	Animals	Total Serum Cholesterol Conc., Mg./100 Cc.	Total Serum Lipid Conc., Mg./100 Cc.	Animals	Neutral Reducing Lipid Excretion,* γ/100 Gm. B. Wt./24 Hr.
Control	23	0	11	40.2	476	12	29.4
NTG-N	12	0	12	55.1	443	0	....
NTG-A	20	+	13	64.3	539	4	59.7
NTG-B	26	+	21	91.3	727	3	120.5
NTG-C	20	+++	15	136.8	1,183	3	104.6
NTG-D	2	+++	2	139.4	1,328	0	....

* The values are taken from a previous publication.[4]

with a drop in the excretion rate. It is interesting to note that the competency of creatinine excretion, which probably depends upon glomerular filtration in the rat,[22] was not affected in these experiments, except in Group D. However, urea excretion, in which tubular function is an important factor, was severely impaired. In addition, Group C, with most retention of urea and chloride, also had diminished water excretion, ascites, and hydrothorax,

Lipemia.—No visible lipemia was observed in the control group or in Group N. There was a slight visible lipemia in Groups A and B, with a conspicuous visible lipemia in Groups C and D (Table 5). There was a progressive rise in the total serum cholesterol concentration, as well as in the total serum lipid concentration, and this reached significant proportions, roughly double the control values, even in Group B, in which hardly any visible lipemia was evident. The cholesterol and lipid concentrations rose in approximately equal ratio, with the principal absolute increase in the noncholesterol lipid fraction. The development of hypercholesteremia, with a parallel increase in the serum phospholipids, had been noted in patients given cortisone and corticotropin (ACTH) by Adlersberg, Schaefer,

22. Friedman, M.: Am. J. Physiol. **146**:387, 1947.

and Drachman.[23] Visible lipemia resulting from the administration of cortisone has been noted in rabbits by Kobernick and More,[24] as well as by Rich, Cochran, and McGoon.[25] It seems reasonable to suggest that in our experiments the visible lipemia is associated with increased adrenal cortical activity.

Neutral Reducing Lipid Excretion.—As previously stated in a preliminary report,[4] the neutral reducing lipid excretion showed a definite increase in the animals with nephritis, roughly parallel to the severity as we have graded it. We have considered the increase in neutral reducing lipid excretion to suggest increased adrenal cortex function. Recent work indicates that this belief may be incorrect.[26]

Gross Anatomy of the Organs.—In the control group, no gross abnormalities were noted in any of the organs, with the exception of the occasional hydronephrosis which occurs in our colony and has been mentioned previously.[1]

Enlargement of the adrenal glands was often grossly noticeable in Groups B to D, but the appearance was otherwise normal. Enlargement of the heart was sometimes grossly noticeable in Groups C and D, but the configuration appeared normal. Gross enlargement of the liver was also noticeable in Groups B to D. The parenchyma seemed paler than normal, and the liver edges were somewhat blunted. However, the greasy feeling associated with fat deposition was absent. The markings appeared normal.

In Groups N and A the kidneys were not grossly different from those of the control group. In Group B the kidneys were obviously enlarged and pale, with a granular surface. The capsule stripped easily. On sagittal section the cortex was pale but of normal width. Petechiae were visible on the surface and in the cortical substance. The corticomedullary junction appeared fairly well defined. In Groups C and D some of the kidneys were like those in Group B, but the manifestations of disease were severer. The kidneys were very much enlarged and very pale, with many petechiae seen on the granular surface. These were pale, depressed surface areas that resembled connective tissue scars. On sagittal section the cortex was thin and the corticomedullary junction poorly defined. The depressed surface areas were connected with the medullary region by radial streaks of pale tissue, and in places there were radial hemorrhagic-appearing areas.

Microscopic Anatomy of the Adrenal Glands.—Except for the differences in cross-sectional area which accompanied the increase of adrenal gland size in Groups B to D, no significant differences between groups were noted in sections stained with hematoxylin and eosin, Mallory's connective tissue stain, and Sudan Black B. The lipid stained with Sudan Black B was variable within each group but had approximately the same range of variability in all groups.

Microscopic Anatomy of the Kidneys.—As previously described,[1] in the control group there were minor anatomic changes when the control kidneys were compared

23. Adlersberg, D.; Schaefer, L. E., and Drachman, S. R.: J. A. M. A. **144**:909, 1950.

24. Kobernick, S. D., and More, R. H.: Proc. Soc. Exper. Biol. & Med. **74**:602, 1950.

25. Rich, A. R.; Cochran, T. H., and McGoon, D. C.: Bull. Johns Hopkins Hosp. **88**:101, 1951.

26. Sobel, H.; Marmorston, J.; Greenfeld, H.; Goodman, H. C.; Sellers, A. L.; Whitney, J. E., and Smith, S: The Relation Between the Adrenal and Pituitary Glands and Urinary Excretion of Neutral Reducing Lipids in the Rat, to be published.

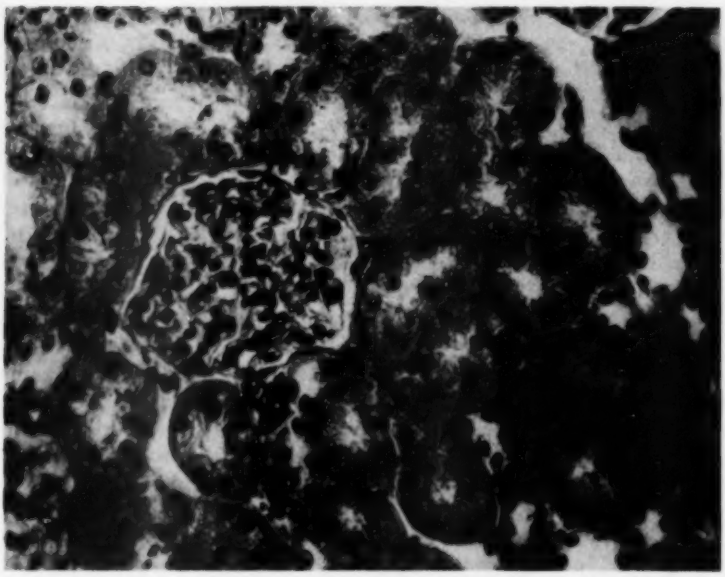

Fig. 3.—Section of rat kidney four weeks after a single intravenous injection of control globulin (GC). Hematoxylin and eosin stain; × 400.

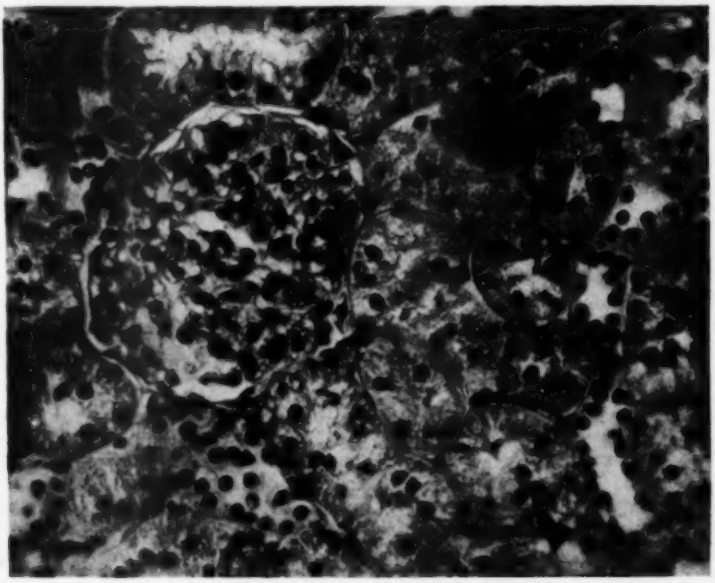

Fig. 4.—Section of rat kidney, Group N, after administration of nephrotoxic globulin. The glomerulus is larger than that seen in Figure 3, and, in the Mallory-stained specimen, there is a significant increase in glomerular collagen. Hematoxylin and eosin stain; × 400.

with normal kidneys from the same colony. The glomeruli were slightly more cellular and contained less blood than normal. In the animals which had been given repeated subcutaneous injections of control globulin (GC) there was a granular eosinophilic precipitate in some of the subcapsular spaces, with very slight evidences of hydropic degeneration in the proximal tubular epithelium. The latter changes were not seen in the animals which had received a single intravenous injection of GC (Fig. 3).

In Group N the kidneys resembled those of the control group very closely. The tubules showed only the slightest evidence of hydropic degeneration, the brush borders were evident, the lumina were free of precipitate and debris and were of

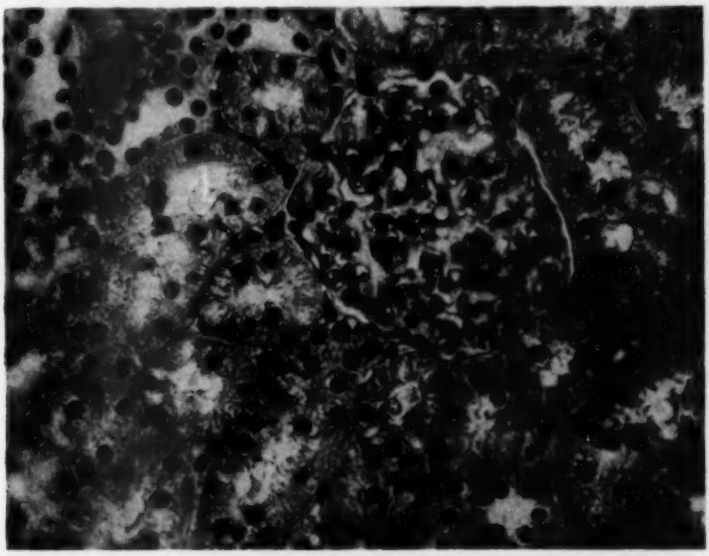

Fig. 5.—Section of rat kidney, Group A, after administration of nephrotoxic globulin. There is an increase in glomerular cellularity. The tubules show ragged cytoplasm, with luminal debris. Hematoxylin and eosin stain; × 400.

normal size (Fig. 4). The glomeruli were slightly more cellular than normal, with less blood than normal. No appreciable precipitate was seen in the subcapsular space. There was a slight increase in the eosinophilic quality of the glomerular interstitium, and, with Mallory's stain, a significantly increased amount of collagen was seen in the glomerular tufts.

In Group A the tubular cytoplasm showed some granularity, with hydropic degeneration, and nuclei of varying size and shape. Debris could be seen in some of the proximal tubules and some of the collecting tubules of the medulla, with occasional eosinophilic precipitates of proteinaceous material in the tubular lumina (Fig. 5). The glomeruli were more cellular than those in Group N, and, especially with Mallory's stain, an increased amount of glomerular collagen could be seen.

There was also an increase in the amount of tubular interstitial collagen. No fibrinoid material was seen in the glomerular tufts, and there were no evidences of inflammatory exudation.

In Group B the tubular damage was quite evident, with granularity of the cytoplasm and a considerable amount of cellular debris in the tubular lumina, some of which were dilated. The nuclei of cells in the proximal convoluted tubules varied in size and shape, as in degenerating cells, but rare mitoses were seen as evidence of regeneration. Precipitates of eosinophilic, proteinaceous material were seen, especially in dilated collecting tubules, both cortical and medullary. The glomeruli contained still more collagen than those in Group A, with distinct thick-

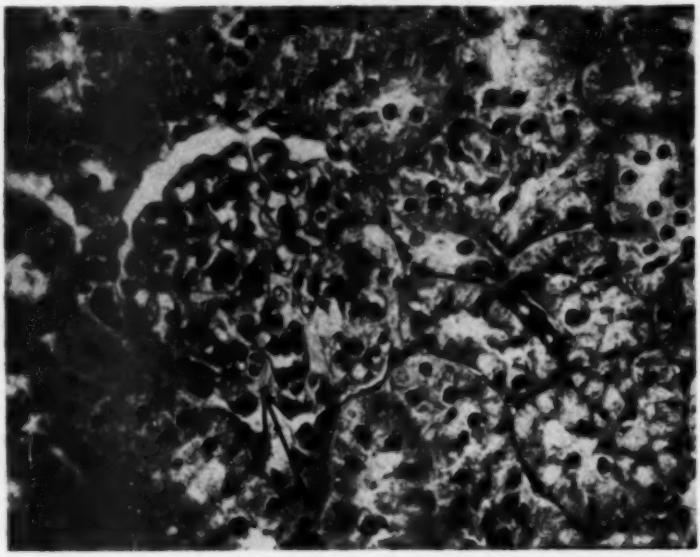

Fig. 6.—Section of rat kidney, Group B, after administration of nephrotoxic globulin. The glomerulus shows more advanced changes, and the arrow points to a cell in mitosis. The tubular nuclei show varying size and staining qualities. Hematoxylin and eosin stain; × 400.

ening of the basement membrane. No fibrinoid deposits were seen. Glomerular proliferation was evident, with rare mitoses seen in the endothelial cells (Fig. 6), increased cellularity of the tuft, and occasional adhesions and epithelial crescents. Evidence of slight glomerular inflammation was seen, with occasional polymorphonuclear leucocytes that were clearly outside the vessel walls.

In Group C all the changes were much more pronounced. The proximal convoluted tubules showed severe damage, many with marked atrophy, lined with low cuboidal epithelium. In other tubules there was marked evidence of hydropic degeneration, with granular cytoplasm, and only patches of the brush border could be seen. The lumina were large (Fig. 7) with much debris and with desquamated

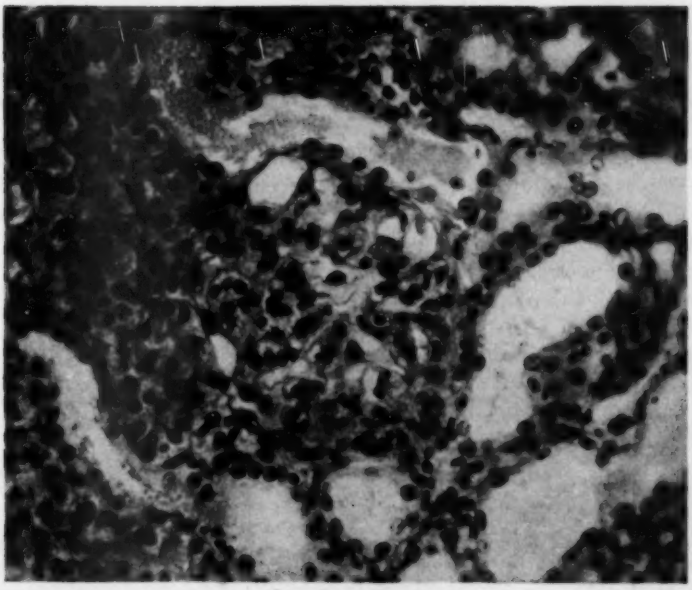

Fig. 7.—Section of rat kidney, Group C, after administration of nephrotoxic globulin. Glomerulus shows capsular adhesions obliterating Bowman's space. Dilated tubules with low cuboidal epithelium and intraluminal proteinaceous precipitate are evident. Hematoxylin and eosin stain; × 400.

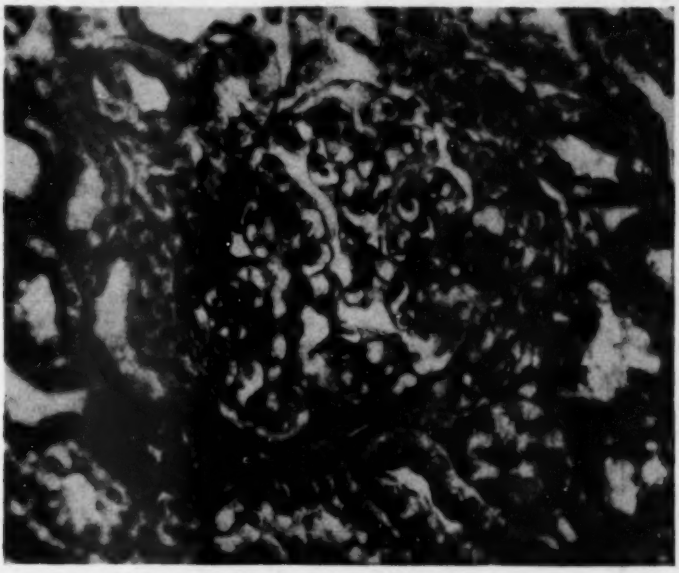

Fig. 8.—Section of rat kidney, Group C, after administration of nephrotoxic globulin. On the left and at the bottom of the glomerulus deeply stained fibrinoid deposits can be seen. On the right is an epithelial crescent. Mallory's connective tissue stain; × 400.

cells. Many tubules contained eosinophilic, proteinaceous precipitate. Nuclei of the proximal convoluted tubule cells showed pyknosis or karyolysis, and in places there were mitoses as evidence of regeneration. The distal convoluted tubules had dilated lumina, but the epithelium seemed better preserved than in the proximal tubules. The glomeruli uniformly showed proliferation, both in the tuft and in the capsule, with many adhesions and epithelial crescents (Fig. 7). Only rarely could the capsular space be seen. The glomerular capillaries were almost obliterated and bloodless. An eosinophilic deposit of fibrinoid material, lying within or upon the capillary loops, was seen in occasional glomeruli (Fig. 8). There was a great

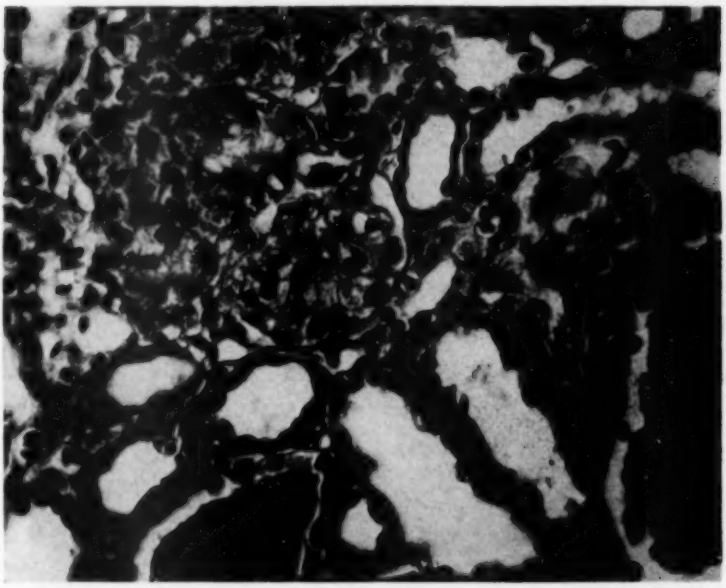

Fig. 9.—Section of rat kidney, Group D, after administration of nephrotoxic globulin. The tubules show enormous dilatation with proteinaceous precipitate. The glomerulus is completely adherent to its capsule with crescent formation. Hematoxylin and eosin stain; × 400.

increase in glomerular collagen, with masses and whorls evident, but none of the glomeruli was completely fibrotic. Thickening of the basement membrane was evident in the glomerular tuft, the capsule, and in the tubular epithelium. Although proliferation was prominent, with many mitoses seen in the glomerular tuft, inflammatory exudate was also present, with infiltration of polymorphonuclear leucocytes, including occasional eosinophilic leucocytes. An increase of interstitial collagen was evident in both cortex and medula.

In the two animals of Group D the changes observed in Group C were all present in even more extreme form. Many enormously dilated distal convoluted tubules, and even collecting tubules, were evident (Fig. 9). The dilated tubules contained highly

eosinophilic, proteinaceous material. The epithelial damage was profound. The glomeruli were similar to those in Group C, but with even more evidence of proliferation and crescent formation. While the fibrinoid material deposited within or upon the capillary loops was prominent, no sclerosed, completely fibrotic glomeruli, such as are seen in human glomerular nephritis, were found. The increase of interstitial collagen was pronounced.

Sections stained with the periodic acid-Schiff technique showed irregular deposition of Schiff-positive material in the basement membrane of glomeruli and tubules. The proteinaceous material in the tubular lumina responded strongly to the Schiff reagent, and in some tubules the brush border and luminal edge of the tubule cells were also Schiff-positive. Serum albumin is considered by some investigators to contain hexose and hexosamine,[27] and serum globulin fractions all appear to contain carbohydrate.[28] Since the proteinaceous material in the tubular lumina almost certainly is composed of serum protein in large part, if not entirely, it is difficult to attach special significance to the Schiff-positive material in the basement membranes.[29] Such material may be related to accelerated passage of serum proteins through the glomerular membrane and, by absorption, through tubule cells, accompanying the proteinuria of nephrotoxic globulin nephritis. It is interesting to note that Smith and associates[30] have observed morphologically similar local concentrations of Evans blue in the brush border and luminal edge of tubule cells, during renin proteinuria after intravenous injection of Evans blue in the rat. This observation was attributed to the tubular absorption of an Evans blue-serum albumin complex.

Sudan III stain showed the presence of rare, scattered deposits of lipoid in Group C, in glomerular tufts and in the tubules. In Group D lipoid deposits were readily seen, but not striking in amount. In none of the groups were any significant changes noted in the blood vessels when these were compared with those of the control groups.

It is of interest to note that in Group N all the physiological data obtained and all gross anatomic observations of the organs were not significantly different from the control data. However, the stained sections could be distinguished from those of the control groups by the deposition of glomerular collagen.

SUMMARY

A system was used for grading the severity of nephritis produced in the rat by injected nephrotoxic globulin (NTG). The grading was based upon relative renal and cardiac size, rate of protein excretion, ascites, and visible lipemia.

When animals were graded by this arbitrary system four weeks after NTG administration, relative adrenal gland weight and neutral reducing lipid excretion

27. Kekwick, R. A.: Biochem. J. **32**:552, 1938. Blix, G.; Tiselius, A., and Svensson, H.: J. Biol. Chem. **137**:485, 1941.

28. Tiselius, A.: Biochem. J. **31**:313 and 1464, 1937. Tiselius, A.: Tr. Faraday Soc. **33**:524, 1937.

29. Preliminary experiments in this laboratory have shown that the serum and urinary proteins of the rat with nephrotoxic globulin nephritis cannot be distinguished from normal serum proteins by electrophoretic characteristics.

30. Smith, S. S., III; Marmorston, J.; Goodman, H. C., and Sellers, A. L.: To be published.

increased with the severity of the nephritis. In addition, liver weight, serum cholesterol concentration and serum total lipid concentration increased in a parallel manner. The increase in blood pressure was only slight in the most severely affected groups.

The increment in relative liver weight was directly proportional to the rate of protein excretion. This circumstance suggested that the hepatomegaly was a result of work hypertrophy. In these animals the liver was required to synthesize a daily increment of serum protein in order to compensate for urinary protein excretion. This daily increment was sometimes three times as much as the normal total circulating protein.

In the groups with severest nephritis the relative weight of the testes was diminished, possibly as the result of increased adrenal activity, with the secretion of androgenic hormones.

STUDIES ON EXPERIMENTAL HYPERTENSION AND CARDIOVASCULAR DISEASE

I. A Method for the Rapid Production of Malignant Hypertension in Bilaterally Nephrectomized Dogs

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CLEVELAND

IN A REVIEW of experiments on renal hypertension in 1937, Goldblatt [1] reported that until that time all observers had failed to produce hypertension by bilateral nephrectomy. Work by Winternitz and associates [2] (1940) gave added confirmation to these observations. When the artificial kidney and peritoneal lavage were developed, Grollman, Muirhead, and Vanatta [3] used these methods to maintain bilaterally nephrectomized dogs for periods up to 69 days and reported the occurrence of hypertension and arterial necrosis in 28 of 38 animals. Leonards and Heisler [4] repeated the work of Grollman and his associates and found that the animals which retained part of the lavage fluid became hypertensive, while those that did not retain the fluid remained normotensive.

In view of the latter observations it seemed a reasonable hypothesis that if fluid were introduced into the peritoneal cavity of bilaterally nephrectomized dogs the result might be a rapid production of hypertension and vascular lesions. To test this hypothesis, the following experiment was performed:

METHODS AND MATERIALS

The dogs were obtained from the pound and were selected only for their general appearance of health and for the ease with which they could be trained for determination of blood pressure. All of these blood pressure determinations were made by direct puncture of the femoral artery and were recorded as mean blood pressure in millimeters of mercury.

From the Institute of Pathology, Western Reserve University.

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1. Goldblatt, H.: Studies on Experimental Hypertension: V. The Pathogenesis of Experimental Hypertension Due to Renal Ischemia, Ann. Int. Med. **11**:69, 1937.

2. Winternitz, M. C.; Mylon, E.; Waters, L. L., and Katzenstein, R.: Studies on the Relation of the Kidney to Cardiovascular Disease, Yale J. Biol. & Med. **12**:623, 1940.

3. Grollman, A.; Muirhead, E. E., and Vanatta, J.: Role of the Kidney in Pathogenesis of Hypertension as Determined by a Study of the Effects of Bilateral Nephrectomy and Other Procedures on the Blood Pressure of the Dog, Am. J. Physiol. **157**:21, 1949.

4. Leonards, J. R., and Heisler, C. R.: Blood Pressure of Bilaterally Nephrectomized Dogs, Federation Proc. **11**(No. 1, Pt. 1):247, 1952.

Two different salt solutions were used in the two groups of experimental animals. One was a sterile 0.9% sodium chloride solution and the other a solution of electrolytes (Skeggs and Leonards [5]) as modified by Leonards. Hereafter, this solution will be referred to as the electrolyte solution to distinguish it from the 0.9% NaCl solution. This particular electrolyte solution was chosen because it closely approximates the concentrations of the major ions in the interstitial fluid of the dog, and has the following ionic composition:

Na^+	148 mEq./L.	Cl^-	126 mEq./L.
K^+	3 mEq./L.	HCO_3^-	24 mEq./L.
Ca^{++}	5 mEq./L.	HPO_4^{--}	2 mEq./L.
Mg^{++}	3 mEq./L.	Lactate	7 mEq./L.
	Glucose	100 mg./100 ml.	

The solution was made up as two stock solutions: A and B.

Solution A		Solution B	
KCl	9.1 gm./L.	$NaHCO_3$	84.0 gm./L.
$CaCl_2$	11.1 gm./L.	Na_2HPO_4	5.7 gm./L.
$MgCl_2 \cdot 6H_2O$	12.2 gm./L.	NaOH	9.6 gm./L.
NaCl	209.0 gm./L.		
Glucose	40.0 gm./L.		
Lactic acid	27.1 ml. (85-90% soln.)		

Each solution was then divided into 25-ml. aliquots, and the aliquots were autoclaved at 20 lb. (9 kg.) pressure for 20 minutes. To make up the final solution, a 25-ml. aliquot of solution A was diluted to approximately 900 cc., solution B was added slowly, and the final dilution to 1 liter was then completed. This procedure was devised to overcome the tendency of solutions A and B to form a precipitate when mixed.

PROCEDURE

In this experiment 25 dogs were used. Blood pressure was determined three times each week until a stable control level was established. In the nephrectomized animals, the nephrectomies were performed one to two weeks apart.

In the first part of the experiment 14 bilaterally nephrectomized dogs were used. Of these, one group of seven dogs was given no food or water after the second nephrectomy and served as the control group. Each of the seven dogs in the other group was given a daily intraperitoneal injection of 100 ml. of 0.9% NaCl solution per kilogram of body weight after the second nephrectomy.

In the second part of the experiment seven bilaterally nephrectomized dogs and four normal dogs were used. Each of the seven bilaterally nephrectomized dogs was given a daily intraperitoneal injection of 100 ml. of the electrolyte solution subsequent to the second nephrectomy. Each of the four normal animals was given a daily intraperitoneal injection of 100 ml. of the electrolyte solution per kilogram of body weight for a period of seven days, and acted as the control group for the experiment.

The animals were thus divided into two control groups and into two experimental groups. The control groups consisted of (1) a group of bilaterally nephrectomized dogs given no fluid throughout the period of survival, and (2) a group of non-nephrectomized dogs given 100 ml. of the electrolyte solution intraperitoneally per kilogram per day for seven days. The two experimental groups consist of (1) a group of bilaterally nephrectomized dogs given 100 ml. of 0.9% NaCl solution intraperitoneally per kilogram per day, and (2) a group of bilaterally nephrectomized dogs given 100 ml. of the electrolyte solution intraperitoneally per kilogram per day. Twenty-five milligrams of terramycin was given with each daily intraperitoneal infusion.

5. Skeggs, L. T., Jr., and Leonards, J. R.: Studies on the Artificial Kidney: I. Preliminary Results with a New Type of Continuous Dialyzer, Science **108**:212, 1948.

After the second nephrectomy or at the beginning of fluid administration, blood pressure determinations were increased from three times per week to once daily. Autopsies were performed on all animals, and both macroscopic and microscopic examinations were made of the thoracic and abdominal viscera, the skeletal muscles, and the skin.

In addition to the above studies, plasma levels of sodium, potassium, calcium, and chloride were determined during the control period and twice during the experimental period. T-1824 (Evans blue) and inulin "space" determinations and permeability of the skin to T-1824 and India ink were determined during the control period and once during the experimental period.

The results of these electrolyte and fluid studies, along with additional data, will be published in a subsequent report.

RESULTS

In agreement with the observations of others, our bilaterally nephrectomized control dogs (given neither fluid nor food) showed no hypertension except for one dog which had a mean blood pressure of 160 mm. Hg on one day. With this exception, all of the dogs maintained a mean blood pressure of less than 150 mm. Hg (Fig. 1*b*).

In all of the non-nephrectomized control dogs, which were given the electrolyte solution, a slight increase in blood pressure developed. It is to be noted that this elevation occurred near the middle of the experimental period and tended to decrease toward the end of the period. This slight elevation may be explained by the fact that a cat had been loose among the dog cages for two days. At most, however, the changes were slight (Fig. 1*a*).

In contrast with the two groups of control dogs, both groups of experimental animals presented definite elevation of blood pressure, usually within 24 hours of the first intraperitoneal infusion. This increased pressure was sustained until the last few hours of life, when the pressure occasionally fell, but never back to control levels. Although these two groups of animals are qualitatively very similar, it is obvious that the quantitative response is considerably greater in those animals given the electrolyte solution than in those given the same amount of 0.9% NaCl solution (Fig. 1*c* and *d*).

Autopsy of the non-nephrectomized control animals revealed no abnormalities. Similarly, in the bilaterally nephrectomized control animals no changes were seen except in animals 36 and 41, in which a few petechiae were present in the mucosa of the stomach and of the colon.

The lesions observed in the experimental animals were qualitatively the same but varied in degree from one animal to another and from one organ to another in the same animal.

The changes in the heart consisted of occasional petechiae in the epicardium and focal or confluent hemorrhages in the endocardium and inner one-third of the myocardium. Associated with the myocardial hemorrhages were focal, poorly defined, finely granular areas of dull grayish yellow, measuring from 2 to 5 mm. in diameter. The endocardial and myocardial changes were usually seen only in the free wall of the ventricles and particularly over the papillary muscles of the left ventricle. The gross changes in the gastrointestinal tract consisted of petechiae in the mucosa and, in the more marked instances, conspicuous hyperemia and confluent hemorrhage. The serosa also revealed hemorrhages, but these occurred as fine linear red markings in the muscularis, which were visible through the transparent serosa. Submucosal edema, particularly of the stomach, was also a common finding. The

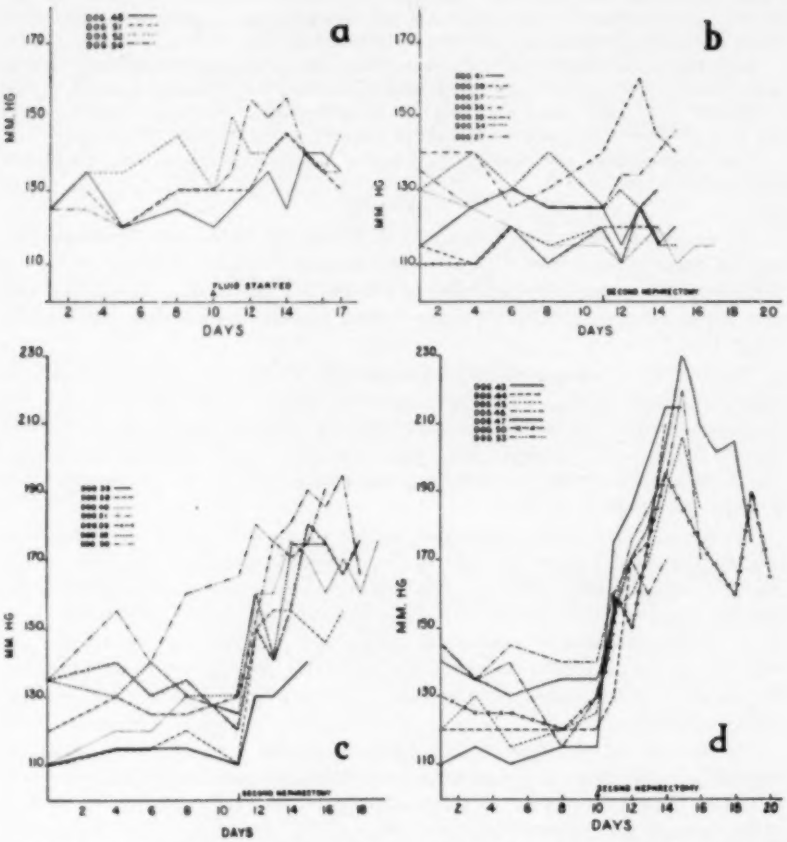

Fig. 1.—(*a*) The mean blood pressure determinations of four normal dogs are presented. From the 10th day to the termination of the experiment, each dog received a daily intraperitoneal injection of 100 ml. of the electrolyte solution per kilogram of body weight.

(*b*) The mean blood pressure determinations of seven dogs are presented. On the 11th day a second nephrectomy was performed on each dog. Subsequent to this procedure, no food or fluid was given throughout the period of survival.

(*c*) The mean blood pressure determinations of seven dogs are presented. On the 11th day a second nephrectomy was performed, and thereafter each dog was given a daily intraperitoneal injection of 100 ml. of 0.9% NaCl solution per kilogram of body weight throughout the period of survival.

(*d*) The mean blood pressure determinations of seven dogs are presented. On the 10th day a second nephrectomy was performed, and after that each dog was given a daily intraperitoneal injection of 100 ml. of the electrolyte solution per kilogram of body weight throughout the period of survival.

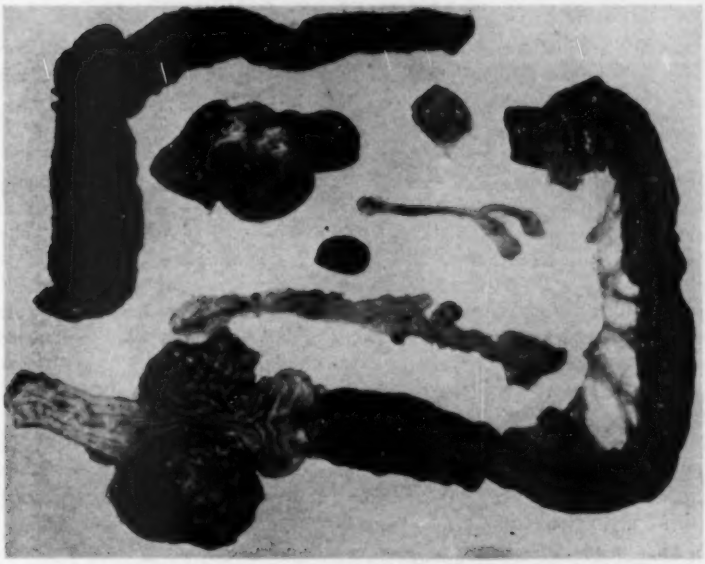

Fig. 2.—Photograph of the organs from Dog 43. The hyperemia and focal hemorrhage in the mucosa of the colon and small intestine, the subserosal and mesenteric hemorrhage of the small intestine, the subendocardial hemorrhage in the heart, and the petechiae of the pancreas and of the mucosa of the stomach, the gall bladder and the urinary bladder are all typical changes in dogs with "malignant" hypertension.

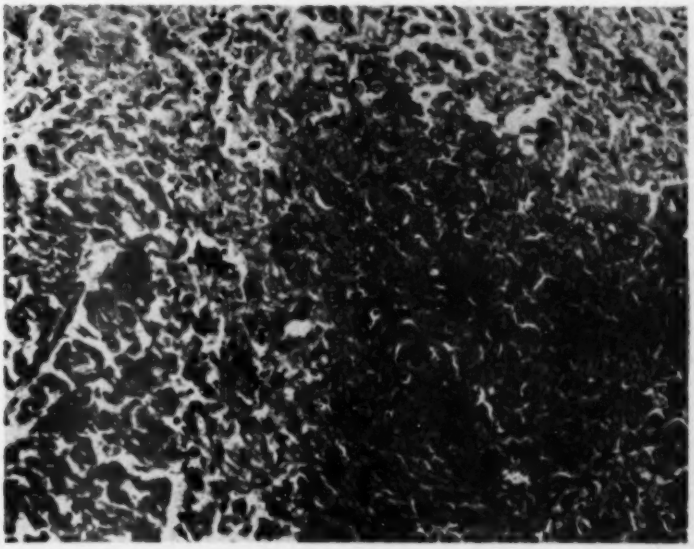

Fig. 3 (Dog 44).—Photomicrograph of a typical myocardial lesion; × 142. On the left there are only slight infiltration of neutrophils and eosinophilia of fibers, while on the right there are marked eosinophilia, necrosis, and homogenization.

pancreas, the adrenal cortex, and the mucosa of the urinary bladder, gall bladder and uterus contained varying numbers of petechiae (Fig. 2). In some instances the hemorrhage in the pancreas was severe, and in these instances the pancreas was very firm, was increased in size, and had a dull, grayish-yellow cut surface. Focal consolidation and hemorrhage were commonly observed in the lungs.

Microscopic examination of the heart revealed the focal discolorations mentioned above to coincide with degenerative changes in groups of muscle fibers. In some of these areas the fibers showed increased eosinophilia and were separated slightly more from one another than in the surrounding normal muscle. In other areas there was a loss of striation in the fibers in addition to the eosinophilia. In still other regions there were interstitial hemorrhage, frank necrosis of muscle, and a moderate infiltration of neutrophils (Fig. 3). These changes were limited almost entirely to the inner one-third of the myocardium. In Dog 50, which survived 10 days after the second nephrectomy, there was proliferation of fibroblasts within occasional areas of myocardial degeneration.

The arterial lesions were similar in all sites but varied in frequency and degree in approximately the following descending order of intensity: stomach, colon, appendix, urinary bladder, small intestine, pancreas, adrenal, gall bladder, esophagus, aorta, and uterine tube.

In some of the arteries and arterioles the lesions were characterized by varying degrees of subendothelial edema and degenerative changes in the media. It is to be emphasized that in these lesions there was no increase in the thickness of the media. In some regions of the vessel wall the nuclei had disappeared, and in others they showed pyknosis and karyorrhexis, all features consistent with necrosis. The cytoplasmic boundaries of the fibers were indistinct and in some areas completely lost, so that the fibrillar pattern of the media became indistinct or unidentifiable (Fig. 4).

In other arteries the wall of the vessel had lost all structural detail and appeared as a homogeneous, intensely eosinophilic mass. The muscle nuclei were usually absent or remained only as an occasional fragment. The wall of the vessel was frequently increased in thickness so that the lumen was constricted. The other striking feature was the homogenization which extended into the paravascular connective tissue so that the external border of the lesion was irregular, poorly defined, and definitely beyond the limits of the vessel (Fig. 5).

Associated with the previously described arterial changes, there was occasionally a slight neutrophilic infiltration of the vessel wall. However, in the animals having the most impressive lesions, this infiltration was sometimes so great that it formed a conspicuous part of the lesion. Under these circumstances there was a necrotic vessel wall surrounded by an intense collection of neutrophils (Fig. 6).

Another lesion occurring in some animals having marked arterial disease was the occlusion of the involved vessels by eosinophilic amorphous or granular masses. In some sites these occlusions were accompanied by endothelial proliferation, which occasionally filled the lumen completely. Although the majority of these were in readily identified arterioles, some were in thin-walled vessels that may have been either veins or aneurysms of arterioles (Orbison[6]). Accurate identification of the site of a lesion like that in Figure 7 depends upon further study by serial section.

6. Orbison, J. L.: Morphology of Thrombotic Thrombocytopenic Purpura with Demonstration of Aneurysms, Am. J. Path. **28**:129, 1952.

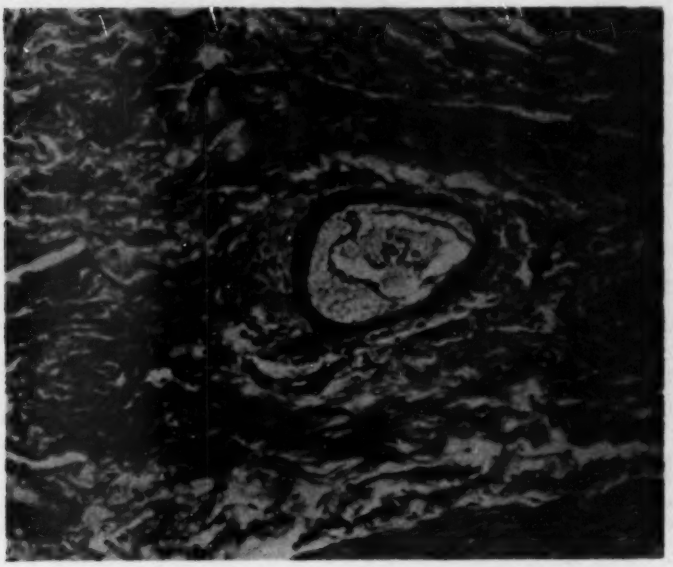

Fig. 4 (Dog 44).—Photomicrograph of an artery in the submucosa of the colon; × 158. The vessel wall has lost all nuclei in some sites and in others shows karyorrhexis. Subendothelial edema is prominent.

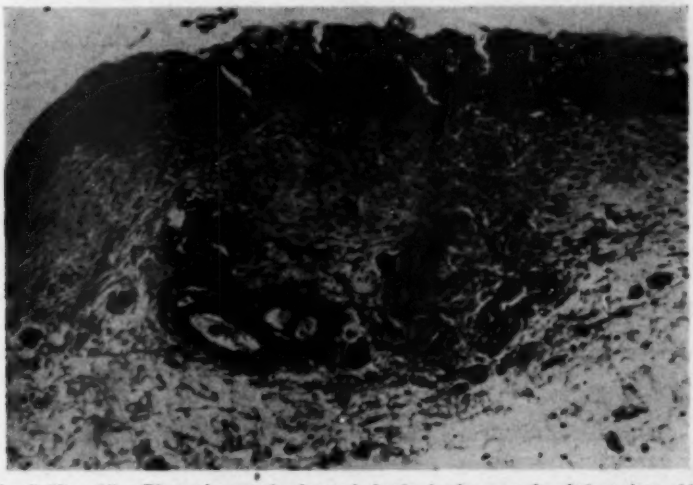

Fig. 5 (Dog 44).—Photomicrograph of vessels in the lamina propria of the urinary bladder to demonstrate the "fibrinoid smudging" of the vessel walls, a change that extends into the perivascular connective tissue; × 130.

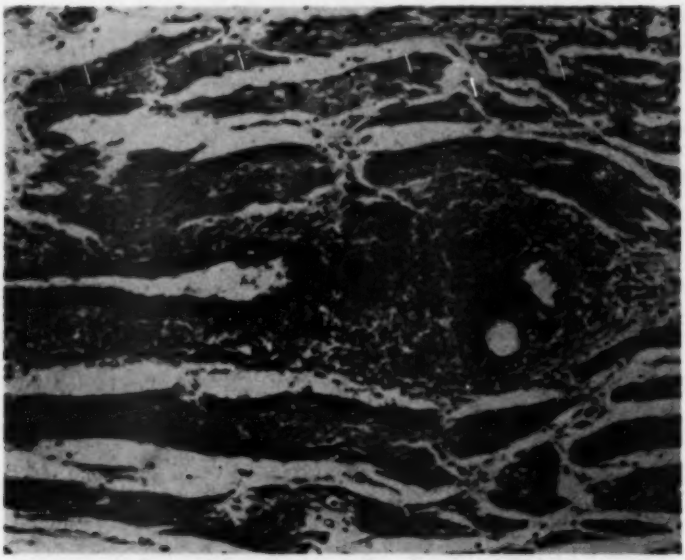

Fig. 6. (Dog 44).—Photomicrograph of arteries in the muscularis of the stomach to illustrate the degree of leucocytic infiltration which occasionally occurs with the necrosis of the vessel wall; × 117.

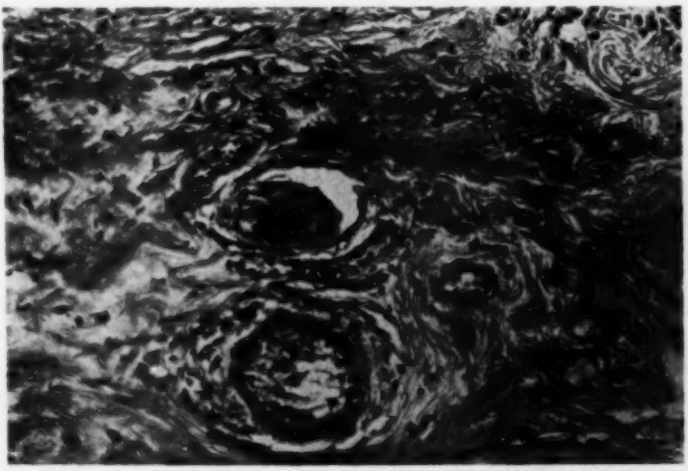

Fig. 7 (Dog 50).—Photomicrograph of vessels in the submucosa of the colon; × 224. One vessel is partially occluded by an amorphous eosinophilic mass, which is covered on its free surface by endothelium. Whether the vessel is an artery or a vein is not demonstrable in this section, but the lesion resembles the arteriolar lesions in thrombotic thrombocytopenic purpura. The other vessel has the typical appearance of a necrotic artery.

The smooth muscle of the gastrointestinal tract, especially the outer muscle layer, frequently had foci of degeneration that consisted of increased eosinophilia, vacuolation of the cytoplasm, separation of fibers from one another, and even necrosis. Hemorrhage was occasionally seen to be associated with these changes, but not as frequently as would have been expected from the gross appearance.

Hemosiderosis of the spleen and definite erythrophagocytosis in the lymph nodes were prominent in the bilaterally nephrectomized animals given fluid. The changes appeared to be equally intense in the dogs given 0.9% NaCl and in those given the electrolyte solution.

Nephrectomized Dogs Given No Fluid	Dog #	27	34	35	36	37	39	41
	Degree of Vascular Lesions	-	-	-	+	-	-	±
Normal Dogs Given Salt Solution IP	Dog #	48	51	52	54			
	Degree of Vascular Lesions	-	-	-	-			
Nephrectomized Dogs Given Normal Saline IP	Dog #	28	29	30	31	32	38	40
	Degree of Vascular Lesions	-	++	++	+	+	-	++
Nephrectomized Dogs Given Salt Solution IP	Dog #	43	44	45	46	47	50	53
	Degree of Vascular Lesions	+++	+++	+++	+++	+++	+++	+++

Fig. 8.—The material presented represents the results of an attempt to quantitate the degree of cardiovascular change in each dog studied. The animals are grouped according to the experimental procedures used. The term "normal saline" refers to 0.9% NaCl solution and the term "salt solution" refers to the electrolyte solution.

Among those dogs which were given the electrolyte solution, large areas of hemorrhage and necrosis of the pancreas occurred in three of seven dogs and their presence is mentioned here only as an interesting incidental observation.

We were unable to identify definite differences in the glomerular zone of the adrenals in control and experimental animals.

A rough quantitation of the arterial lesions was attempted by assigning values from 0 to +++ to the changes in the heart, the stomach, and the colon. By adding the number of pluses for the three organs quantitated, a number was obtained which ranged from 0 to 9. This number was then compared with the following scale to determine a quantitative estimate:

$0 - 1\frac{1}{2} = \pm$; $2 - 4 = +$; $4\frac{1}{2} - 6\frac{1}{2} = ++$; $7 - 9 = +++$

As seen in Figure 8, lesions developed in only two of seven dogs that were nephrectomized and given no fluid, and in those two were only slight. In the normal dogs given the electrolyte solution no lesions developed. In five of the bilaterally nephrectomized dogs given 0.9% NaCl, slight to moderate lesions developed, whereas two had no lesions. Finally, in all of the bilaterally nephrectomized dogs given the electrolyte solution, notable lesions developed. It should be stated that these animals did not all have conspicuous lesions in all three "quantitated" sites but that all of the lesions were sufficiently great so that when considered together, they fell into the +++ group.

COMMENT

Repeated attempts to produce hypertension by bilateral nephrectomy had failed until 1947, when Braun Menendez and von Euler [7] reported the occurrence of hypertension in bilaterally nephrectomized rats. However, it was not until 1949 that Grollman and associates [3] produced hypertension in bilaterally nephrectomized dogs kept alive by peritoneal lavage. He interpreted his results as indicating that one of the activities of the kidney is to prevent hypertension and that when this activity was lost by bilateral nephrectomy hypertension developed if the animals could be maintained for a sufficient period of time.

Leonards and Heisler [4] repeated the work of Grollman and associates and found that the hypertension depended upon the degree of hydration of the animals. By determining inulin "space" and T-1824 "space," they concluded that the hypertension varied directly with the increase or the decrease of "interstitial" fluid volume.

These results suggested that administering large quantities of fluid to bilaterally nephrectomized dogs would produce hypertension in a short period of time. This proved to be true. However, since we had used 0.9% NaCl solution as our infusion fluid, the possibility was considered that the result might be the effect of the sodium ion rather than the effect of the fluid.

In an attempt to control the effect of increased sodium ion alone, we repeated the experiment, substituting the electrolyte solution containing the major electrolytes in concentrations similar to those of the interstitial fluid of the dogs. This solution proved to be even more effective in the production of both hypertension and vascular lesions than was the NaCl solution alone.

These results made it apparent immediately that more detailed studies of fluid and electrolyte balance were necessary before conclusions could be made regarding the mechanism involved in these changes. However, the fact that the electrolyte solution caused greater increases in blood pressure and greater vascular changes than did the sodium chloride solution suggests that the composition of the fluid plays a definite role in the pathogenesis of both the hypertension and the vascular disease. Furthermore, the possibility must be considered that some constituent of the electrolyte solution has at least a potentiating effect on the sodium chloride. Definite information on these points must await more detailed studies.

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7. Braun Menendez, E., and von Euler, U. S.: Hypertension after Bilateral Nephrectomy in Rat, Nature, London **160**:905, 1947.

The results presented here do not correspond well with Grollman's original hypothesis that hypertension in bilaterally nephrectomized dogs results from the loss of a hypertension-preventing effect of the kidney. If this were the case, then hypertension should develop in all of the bilaterally nephrectomized dogs to approximately the same degree and within approximately the same interval. Since this did not occur, and since fluid and electrolyte caused rapid onset of hypertension, our impression at the present time must be that fluid and electrolyte are the principal known factors influencing blood pressure and vascular lesions in bilaterally nephrectomized dogs. In a more recent report, Turner and Grollman [8] suggest the possibility of failure of a mechanism controlling the ratio of sodium to potassium between the cells and the interstitial fluid.

Logically, the question arises whether the phenomenon reported here is governed by the same mechanisms as the experimental hypertension with arterial necrosis observed following various manipulations of the kidney and the giving of desoxycorticosterone acetate and saline. Since the ultimate mechanisms of hypertension and arterial necrosis are not known, only inferences concerning this subject can be drawn, and these depend, in this study, upon histological data. The parallelism between the lesions in our animals and those reported by Muirhead and co-workers [9] is complete except that we did not find the "chronic" vascular lesions and the organized myocardial lesions which they reported in those animals surviving for longer periods. This parallelism includes even the disturbances of the hematopoietic system, characterized by anemia, hemosiderosis of spleen and liver, and erythrocyte phagocytosis in the lymph nodes.

The similarity which the lesions we produced bore to those produced by Goldblatt [10] in dogs is likewise striking, although subintimal hyalinization was not frequent in our animals. The similarity noted between the lesions of our animals and those of the desoxycorticosterone acetate- and saline-treated animals is not as apparent, and the additional complication of species difference enters into consideration, since the desoxycorticosterone and saline work has been principally in rats.

It is apparent, then, that the histological alterations occurring in hypertension produced by several methods are similar and thus suggest a similar pathogenesis. However, it must be reemphasized that such a suggestion is based upon the assumption that the histological lesions can result from only one mechanism and that the hypertension and vascular lesions are the result of the same mechanism. Since no evidence exists to establish these assumptions as facts, we cannot state that the hypertension and vascular lesions resulting from these various procedures are the same. We can only suggest the possibility of a similar underlying pathogenesis.

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8. Turner, L. B., and Grollman, A.: Role of Adrenal in Pathogenesis of Experimental Renal Hypertension as Determined by a Study of the Bilaterally Adrenalectomized and Nephrectomized Dog, Am. J. Physiol. **167**:462, 1951.

9. Muirhead, E. E.; Grollman, A., and Vanatta, J.: Hypertensive Cardiovascular Disease ("Malignant Hypertension"): Changes in Canine Tissues Induced by Various Manipulations of the Kidney, with Special Reference to Vascular and Myocardial Lesions, Arch. Path. **50**:137, 1950.

10. Goldblatt, H.: Studies on Experimental Hypertension: VII. The Production of the Malignant Phase of Hypertension, J. Exper. Med. **67**:809, 1938.

SUMMARY

Hypertension was consistently produced in bilaterally nephrectomized dogs within 24 to 48 hours after the second nephrectomy by the intraperitoneal injection of either 0.9% sodium chloride solution or the electrolyte solution of more complex composition. That this hypertension was "malignant" is indicated by the fact that 12 of 14 dogs had arterial necrosis and myocardial hemorrhage and necrosis.

In these animals the development of hypertension was not caused by the presence of a renal pressor substance, since both kidneys had been removed, or by the absence of a hypertension-suppressing substance of renal origin, since bilateral nephrectomy alone failed to produce the hypertension.

The electrolyte solution was found to produce a greater degree of hypertension and more pronounced vascular disease than the 0.9% NaCl solution alone. This indicates that the amount of fluid is not the only factor involved in this phenomenon and that the composition of the fluid also plays a role.

The histological alterations in these animals are similar to those described in other reports on "malignant hypertension" and suggests the possibility of a similar underlying mechanism.

EFFECT OF BIOFLAVONOIDS ON RADIOSENSITIVITY OF TRANSPLANTED TUMORS

Effect on Tolerance to Contact Radiation

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AND
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SOKOLOFF and associates [1] demonstrated that a vitamin-P-like (bioflavonoid) compound extracted from citrus waste gives considerable protection to rats submitted to a sublethal total-body dose of radiation and reduces capillary fragility induced by leukotaxine and bacterial polysaccharide. Since then, the vitamin-P-like compound has been tested clinically by Arons and associates,[2] Miller, Hurt, Davis, and others [3] and it has been found that the tolerance of cancer patients submitted to x-ray irradiation is greater when the vitamin-P-like compound is administered simultaneously, as compared with that of controls. This was evidenced by a considerable decrease or a complete absence of radiation erythema. Since the vitamin-P-like compound is now being used by radiotherapists mostly in the x-ray therapy

From the Southern Bio-Research Laboratory, Florida Southern College.

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1. Sokoloff, B.; Redd, J. B., and Dutcher, R.: Science **112**:112, 1950; Quart. J. Florida Acad. Sc. **12**:185, 1950; Proc. Soc. Exper. Biol. & Med. **75**:6, 1950; Federation Proc. **2**(No. 1):344, 1950; Bull. Fifth Internat. Cancer Cong., Paris, July, 1950. Sokoloff, B.; Eddy, W. H., and Redd, J. B.: J. Clin. Invest. **30**:395, 1951.

2. Arons, I.; Freeman, J., and Oppenheim, A.: Cancer Res. **11**:234, 1951; Bull. Fifth Internat. Cancer Cong. Paris, July, 1950; Sixth Internat. Cong. of Radiology, London, July, 1950.

3. Personal communications from Martin S. Abel, The Permanente Hospitals, Oakland, Cal.; P. A. Bishop, Pennsylvania Hospital, Philadephia; Harold E. Davis, Jackson Memorial Hospital, Miami; P. R. Dirkse, St. Francis Hospital, Peoria, Ill.; W. A. Gore, Wadsworth General Hospital, Los Angeles; I. E. Hummon, Cook County Hospital, Chicago; F. K. Hurt, St. Vincent Hospital, Jacksonville, Fla.; Ivan Isaacs, Duval Medical Center, Jacksonville, Fla.; Arnold H. Janzen, Yale University Medical School; J. Cash King, Methodist Hospital, Memphis; William P. Martin, Buffalo General Hospital, Buffalo; Theodore Miller, Pack Medical Group, New York; Walter T. Murphy, Roswell Park Memorial Institute, Buffalo; Raymond E. Parks, Jackson Memorial Hospital, Miami; C. B. Rossignol, St. Luke's Hospital, New York; Lester A. Smith, Indianapolis General Hospital; Leslie Sycamore, Mary Hitchcock Memorial Hospital, Hanover, N. H., and Edward L. Voke, The Peoples Hospital, Akron, Ohio.

of cancers, it was found advisable to investigate the possible effect of bioflavonoids on cancerous growth. In a recent publication, Sokoloff and co-workers [4] reported that the bioflavonoid compound has a moderate inhibitory effect on Carcinoma 175-G, Crocker rat carcinoma, and mouse sarcoma 180 without causing any ill-effect in the animals. The present investigation concerns the effect of the bioflavonoid compound on the radiosensitivity of transplanted tumors.

MATERIAL AND TECHNIQUE

Two transplanted tumors were chosen for this investigation: Rat Sarcoma 39 and Crocker (August) rat carcinoma. While the first of these tumors shows a tendency toward spontaneous disappearance (in about 8%), the second tumor is very virulent and not a single disappearance has been noted in our stock of these animals during the last five years. In both instances we took tumors 17 to 19 mm. in diameter and of approximately the same weight and age. August rat carcinoma grows faster, and a diameter of 17 to 18 mm. was usually reached in 10 to 12 days, while it took about 15 days for Sarcoma 39 to reach the same size. The Chaoul contact apparatus served us for radiation experiments. The radiation factors were 60 kv., a 0.12-mm. Cu filter, and a 3-cm. distance from the target to the center of the tumor. This was obtained by slight pressure of the applicator on the surface of the tumor. The field was 2 cm. in diameter with 450 r/min. This was measured with a Siemens dosimeter, carefully standardized. In the present work, only a single dose of radiation was applied, and therefore the time-intensity factor was not investigated.

Four single doses of contact radiation were investigated in connection with the effect of flavonoids: 6,000, 10,000, 12,000, and 15,000 r. From our previous work, when a single dose of total-body radiation is applied, the protective action of flavonoids against radiation injury appears more pronounced when the flavonoid compound is administered for 5 to 7 days prior to and for 15 to 20 days after the exposure than when it is administered only after the exposure.[5] This observation suggested the necessity of similar experiments with the contact irradiation of tumors. Thus our experiments concern the effect of administering flavonoids (*a*) before and after the exposure to radiation, (*b*) only after the exposure, and (*c*) only before the exposure.

A bioflavonoid compound isolated from citrus waste, similar to but not identical with the original "citrin" of Szent-Gyorgyi, was used in this investigation. The chemical nature of this compound was described elsewhere.[6] In all experiments a daily dose of 10 mg./100 gm. wt. was given to animals for 28 days, or a total dose of 280 mg./100 gm. wt. This dose produces no toxic effect, as was demonstrated by us on normal rats. It corresponds, if measured in weight, to a daily dose of 0.8 to 1.0 gm. of the flavonoid compound given to cancer patients during the course of radiation therapy.

EXPERIMENTS

(*a*) *Flavonoids Administered Prior to and After Radiation.*—In the first series of experiments a dose of 6,000 r contact radiation was applied. Fifty rats, bearers of Sarcoma 39, and 50 rats, bearers of Crocker carcinoma, were used for this trial. In each group of animals 25 rats served as controls, receiving no flavonoids, and the rest, 25 rats, were given 10 mg./100 gm. wt. daily, 7 days prior to and 21 days after

4. Sokoloff, B.; Eddy, W. H.; Williams, J., and Sciortino, L.: Cancer Res. **11**:283, 1951; Federation Proc. **10** (No. 1):354, 1951. Sokoloff, B.; Eddy, W. H., and Redd, J. B.: A. M. A. Arch. Path. **52**:210, 1951.

5. Sokoloff, B.: Research in Flavonoids, Report, Contract NR 122-082, Office of Naval Research, U. S. Navy Department, 1952, to be published.

6. Sokoloff, B.; Eddy, W. H., and Redd, J. B.: J. Clin. Invest. **30**:395, 1951. The flavonoid compound contained four identified factors, namely: hesperidin-chalcone, glucose-hesperidin chalcone, eriodictyol, and a quercitrin-like substance, which seemed to form a complex molecule as U. V. readings have shown.

radiation. The animals tolerated this dose of radiation quite well and there was no mortality in either group of rats. Table 1 summarizes the results of this trial.

The results so obtained indicate that Sarcoma 39 is more sensitive to this dose of contact radiation than Crocker carcinoma. A single dose of 6,000 r completely destroyed Sarcoma 39 in 59% of rats and Crocker carcinoma in only 22%. There was no apparent difference in radiosensitivity between the group of animals receiving flavonoids and the control animals.

TABLE 1.—*Contact Radiation, 6,000 R*

Type of Tumor	Average Diameter of Tumor, Mm.	Animals	Total Dose of Flavonoids, Mg./100 Gm. Wt.	Tumors Completely Destroyed, %
Sarcoma 39	19	25	280	65
Sarcoma 39	19.3	25	None	59
Crocker carcinoma	18.6	25	280	21
Crocker carcinoma	19.2	25	None	22

TABLE 2.—*Contact Radiation, 10,000 R*

Type of Tumor	Average Diameter of Tumor, Mm.	Animals	Total Dose of Flavonoids, Mg./100 Gm. Wt.	Tumors Completely Destroyed, %	Mortality from Exposure, % *	Mortality from Advanced Cancer, % †
Sarcoma 39	19.8	25	280	96	4.0	0
Sarcoma 39	18.4	25	None	100	0	0
Crocker carcinoma	19.6	25	280	64	0	36
Crocker carcinoma	18.2	25	None	56	4.0	40

* The death occurred within 10 to 12 days after the exposure, from radiation illness.
† The deaths occurred 30 to 35 days after the exposure, from advanced growth.

TABLE 3.—*Contact Radiation, 12,000 R*

Type of Tumor	Average Diameter of Tumor, Mm.	Animals	Total Dose of Flavonoids, Mg./100 Gm. Wt.	Tumors Completely Destroyed, %	Mortality from Exposure, % *	Mortality from Advanced Cancer, % †
Sarcoma 39	19.9	18	280	100	0	0
Sarcoma 39	18.3	18	None	100	16.65	0
Crocker carcinoma	19.2	18	280	72.2	5.5	27.8
Crocker carcinoma	17.9	18	None	77.8	22.3	22.3

* The deaths occurred within 12 to 14 days, from radiation illness.
† The deaths occurred 30 to 36 days after the exposure, from advanced growth. In the flavonoid group of Crocker carcinoma, five rats, or 27.8%, died from advanced cancer. One rat, or 5.5%, with a tumor destroyed by radiation died from radiation illness. In the nonflavonoid group of rats with Crocker carcinoma four rats, or 22.3%, died of advanced cancer. Four rats, or 22.3%, with tumors destroyed by radiation died of radiation illness.

In the next series of experiments a dose of 10,000 r contact radiation was delivered. Fifty rats bearing Sarcoma 39 and 50 rats bearing Crocker carcinoma were used for the trial. This dose of radiation was well tolerated by the animals, and only two rats died after the exposure. Table 2 gives the summary of this trial.

In the third series of experiments a dose of 12,000 r was applied. Thirty-six rats with Sarcoma 39 and 36 rats with Crocker carcinoma served for this trial. Table 3 gives the summary of these experiments.

The mortality from radiation was higher in the rats that were kept on the regular diet without flavonoid than in the group given flavonoid. There was no difference in radiosensitivity of either Sarcoma 39 or Crocker carcinoma between the treated and the control groups.

In the next series of experiments a dose of 15,000 r contact radiation was applied. This dose caused high mortality among the rats. The deaths occurred at various times after the exposure. Some animals succumbed on the 15th day after irradiation, while others lived as long as 22 to 23 days. Table 4 summarizes the results when 15,000 r was delivered to rats that were bearers of Sarcoma 39 and rats that were bearers of Crocker carcinoma. Altogether 100 rats were used for this trial. The trial demonstrated the protective action of flavonoids against radiation injury. The destruction of tumors was as complete in the flavonoid as in the control group.

TABLE 4.—*Contact Radiation, 15,000 R*

Type of Tumor	Average Diameter of Tumor, Mm.	Animals	Total Dose of Flavonoids, Mg./100 Gm. Wt.	Tumors Completely Destroyed, %	Mortality from Exposure, %*	Mortality from Cancer
Sarcoma 39	19.8	25	280	100	24	0
Sarcoma 39	18.2	25	None	100	72	0
Crocker carcinoma	20.0	25	280	100	32	0
Crocker carcinoma	19.5	25	None	100	76	0

* The mortality rate refers to deaths from radiation illness. At the time of the rats' death the tumors were absent altogether.

TABLE 5.—*Contact Radiation, 15,000 R*

Type of Tumor	Average Diameter of Tumor, Mm.	Animals	Total Dose of Flavonoids, Mg./100 Gm. Wt.	Tumors Completely Destroyed, %	Mortality from Exposure, %*	Mortality from Cancer
Sarcoma 39	20.0	25	280	100	64	0
Sarcoma 39	19.0	25	None	100	84	0
Crocker carcinoma	20.0	25	280	100	68	0
Crocker carcinoma	18.5	25	None	100	80	0

* The mortality rate refers to deaths from radiation illness. The tumors at the time of the rats' death were absent. Animals which survived were free of cancerous growth.

(*b*) *Flavonoids Administered After the Exposure.*—The dose of 15,000 r contact radiation was chosen for the investigation of the effect of postradiation administration of the flavonoid compound on the survival of irradiated rats. Fifty rats with Sarcoma 39 and 50 rats with Crocker carcinoma served for this trial, the results of which are summarized in Table 5. The flavonoid compound, 10 mg./100 gm. wt., was given daily for 28 days after the exposure. No flavonoids were administered prior to the exposure. The results are given in Table 5. The data are indicative of a relatively slight protective activity of flavonoids against radiation injury when the flavonoid compound was administered after the exposure. The difference between the mortality rates of the treated and the control group is small.

(*c*) *Flavonoids Administered Before the Exposure.*—The same dose of 15,000 r contact radiation was applied to a group of rats receiving the flavonoid compound only before the exposure. No flavonoids were administered the day of the exposure or after it. Fifty rats with Sarcoma 39 and 50 with Crocker carcinoma were used

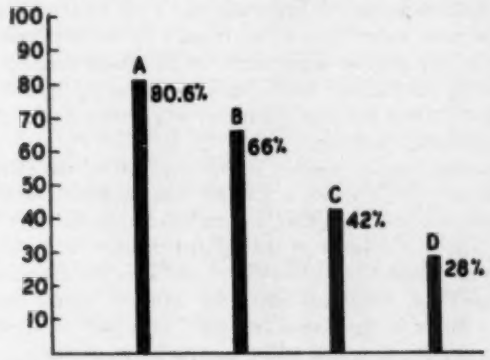
for this trial. The flavonoid compound was administered, 10 mg./100 gm. wt. daily for 28 days prior to the exposure. The summary of this trial is presented in Table 6. The data presented in Table 6 show that the flavonoid compound when administered before the exposure gives considerable protection against radiation injury.

The difference in the protective activity of flavonoids when they are administered before or after the radiation, as well as when they are given before and after the exposure, is illustrated by the figures of the accompanying chart.

TABLE 6.—*Contact Radiation, 15,000 R*

Type of Tumor	Average Diameter of Tumor, Mm.	Animals	Total Dose of Flavonoids, Mg./100 Gm. Wt.	Tumors Completely Destroyed, %	Mortality from Exposure, %*	Mortality from Cancer
Sarcoma 39.....	20.0	25	280	100	44	0
Sarcoma 39.....	18.2	25	None	100	88	0
Crocker carcinoma.....	19.9	25	280	100	40	0
Crocker carcinoma.....	18.6	25	None	100	84	0

* The mortality rate refers to death caused by radiation illness. All tumors were destroyed.



Mortality rates of rats bearing Sarcoma 39 and Crocker carcinoma (average diameter of tumors 18 to 20 mm.) and receiving 15,000 r contact radiation:

A (control, no flavonoids), 80.6%, average for 150 rats

B (280 mg. of flavonoids given after the exposure), 66%, average for 50 rats

C (280 mg. of flavonoids given before the exposure), 42%, average for 50 rats

D (280 mg. of flavonoids administered for 7 days prior to the exposure and for 21 days after the exposure), 28%, average for 50 rats

The analysis of the data presented in the chart leads to the conclusion that it is essential to administer flavonoids prior to the exposure in order to obtain maximal protection against radiation injury. When flavonoids were administered after the exposure, their protective activity was manifested only in a small degree. However, the lowest mortality rates were obtained when flavonoids were given before and after the exposure. In this case the mortality rate for the flavonoid group was 28% while that for the control group, receiving no flavonoids, was 80.6% (average for 150 rats).

COMMENT

Ionizing radiations induce an alarm reaction in the organism of man and animal alike, according to Selye.[7] Such stress reactions have been the subject of an extensive review by Edelmann.[8] Much additional evidence has accumulated to show the extraordinary effectiveness of x-ray radiation in producing what Selye calls the General-Adaptation-Syndrome (Betz and Herve[9]; Ellinger[10]; Sokoloff and Eddy[11]). The gravity and the extension of the alarm reactions depend to a considerable degree on the dose of radiation applied. When the dose of radiation is very large, it may lead to a stage of exhaustion or, if within the limit of the organism's tolerance, to a stage of resistance (Selye). One of the important features of the stress reactions induced by ionizing radiation is the injury to the capillary wall. The intercellular cement of the capillary wall is altered long before injury to the endothelial cells is evidenced. The contours and the size of the pores are modified, and subsequently capillary permeability and fragility are increased. This injury of the capillary wall can be traced in animals submitted to radiation.[12] These findings were corroborated by the work of Boden,[13] Ahlbom,[14] and Stevenson and Eckhardt[15] on radiation myelitis.

Boden stated that in all cases of radiation myelitis investigated by him, the microscopic observations at autopsy suggested that x-ray affected primarily the capillary system of the brain rather than its neurones, and that the eventual neurologic lesions were results of gradual impairment of the blood supply. It is in this respect, in protecting the capillary wall against radiation injury, that bioflavonoids exert their biological action and thus counteract to a certain degree the stress reactions induced by ionizing radiation.

This action is slow, and it requires several days before the effect of flavonoid therapy is manifested. This explains the fact that flavonoids administered after the exposure exert only minimal effect on irradiated animals. Yet since bioflavonoids participate also in restitution of the injured capillary wall, flavonoid therapy gives the best results when it is applied before and after the exposure. As Rekers and Field[16] pointed out, flavonoids affect the capillary system directly, perhaps participating as a factor in the "wear and tear" of a part or all of the capillary system, inhibiting degeneration and taking part in its regeneration.

In spite of the fact that bioflavonoids give protection against radiation injury to the capillary wall, they do not decrease the radiosensitivity of cancer. This might be explained by a fact long ago postulated by F. C. Wood and A. Lacassagne. The vascular system of the cancer, being poorly formed, is much more radiosensitive

7. Selye, H.: Annual Report on Stress, Montreal, Acta Company, 1951.

8. Edelmann, A.: Ann. Rev. Physiol. **12**:27, 1950.

9. Betz, H., and Herve, A.: Compt. rend. Soc. biol. **144**:1015, 1950.

10. Ellinger, F.: Radiology **51**:394, 1948.

11. Sokoloff, B., and Eddy, W. H.: Capillary Fragility and Stress, Monograph 3, Lakeland, Fla., Florida Southern College, 1952.

12. Sokoloff, B., and Redd, J. B.: Capillary Permeability and Fragility, Monograph 1, Lakeland, Fla., Florida Southern College, 1949.

13. Boden, G.: J. Fac. Radiologists **2**:79, 1950.

14. Ahlbom, H. E.: Acta radiol. **22**:155, 1941.

15. Stevenson, L. D., and Eckhardt, R. E.: Arch. Path. **39**:109, 1945.

16. Rekers, P. E., and Field, J. B.: J. Clin. Invest. **28**:746, 1949.

than the capillary system of normal tissue. And, as in the case of cancer cells, the capillary system of a growing tumor is injured by radiation more gravely (beyond any repair) than the capillary system and cells of the surrounding tissues and organs. Thus, apparently, bioflavonoids, while increasing the tolerance to radiation, do not interfere with the destructive action of x-radiation upon cancer tissue. This conclusion seems to be corroborated by the clinical investigations based on the use of the flavonoid compound in radiation therapy, which now covers up to about 500 case histories reported from 22 hospitals.

SUMMARY

The flavonoid compound when given in a total dose of 280 mg./100 gm. wt. to rat bearers of Sarcoma 39 or Crocker carcinoma, of an average tumor diameter of 17 to 20 mm., which were submitted to a single dose of 6,000, 10,000, 12,000, or 15,000 r contact radiation, respectively, did not decrease the radiosensitivity of the cancer tissue.

The flavonoid compound when administered in a total dose of 280 mg./100 gm. wt. to rat bearers of Sarcoma 39 or Crocker carcinoma *after* they had been exposed to a single dose of 15,000 r contact radiation exerted only slight, if any, protective action against radiation injury. The mortality rate for this group of rats was 66% against 82% of the control group of irradiated rats.

The flavonoid compound when administered in a total dose of 280 mg./100 gm. wt. to rat bearers of Sarcoma 39 or Crocker carcinoma *before* they were exposed to a single dose of 15,000 r contact radiation gave considerable protection against radiation injury. The mortality rate for this group of rats was 42% against 86% of the control group of irradiated rats.

The flavonoid compound when given in a total dose of 280 mg./100 gm. wt. to rat bearers of Sarcoma 39 or Crocker carcinoma, for 7 days *prior to* and 21 days *after* they were exposed to a single dose of 15,000 r contact radiation brought down the mortality rate to 28% against 74% for the control group of irradiated rats.

The possible relation between the biological activity of flavonoids and the stress reactions induced by radiation is discussed.

EFFECT OF THYROXIN AND 2,4-DINITROPHENOL ON THE RETROGRESSION OF EXPERIMENTAL ATHEROSCLEROSIS

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AND
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THE ATHEROSCLEROTIC lesions induced in the aortas of rabbits fed a diet enriched with cholesterol had been observed to retrogress slowly when the animals were returned to a normal diet.[1] Several unsuccessful attempts had been made to hasten the spontaneous disappearance of the intracellular and extracellular lipids contained in atherosclerotic lesions.[2] These experiments had demonstrated clearly that procedures which are capable of inhibiting the production of experimental cholesterol atherosclerosis are without effect on the retrogression of the lesions.[3] It was considered that substances such as thyroxin or 2,4-dinitrophenol, which increase the rate of cellular metabolism, might prove to be capable of promoting the catabolic destruction of lipids in atherosclerotic plaques and of hastening retrogression of the plaques. The effect of these two agents on the regression of the aortic lesions of experimental cholesterol atherosclerosis of the rabbit was studied in the following experiment.

PROCEDURES

White rabbits of the New Zealand strain, aged 2½ to 3 months, were fed 1 gm. of cholesterol mixed with 6 gm. of corn oil and 93 gm. of rabbit chow daily for five days each week. The cholesterol consumption was estimated by weighing any uneaten residue. The rabbits were given normal chow on the remaining two days of each week. After a period of 13 weeks of cholesterol feeding the animals were returned to a normal diet. A period of 30 days was allowed to elapse

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This investigation was assisted by a grant from the Canadian Life Insurance Officers Association.

1. Anitschkow, N.: Ueber die Rückbildungsvorgänge bei der experimentellen Atherosklerose, Verhandl. deutsch. path. Gesellsch. **23**:473, 1928.

2. (*a*) Duff, G. L., and Meissner, G. F.: The Effect of Choline on Experimental Atherosclerosis in the Rabbit, to be published. (*b*) Duff, G. L., and McMillan, G. C.: The Effect of Alloxan Diabetes on Experimental Cholesterol Atherosclerosis in the Rabbit: I. The Inhibition of Experimental Cholesterol Atherosclerosis in Alloxan Diabetes; II. The Effect of Alloxan Diabetes on the Retrogression of Experimental Cholesterol Atherosclerosis, J. Exper. Med. **89**:611, 1949. (*c*) Kellner, A.; Correll, J. W., and Ladd, A. T.: The Influence of Intravenously Administered Surface-Active Agents on the Development of Experimental Atherosclerosis in Rabbits, ibid. **93**:385, 1951. (*d*) Meeker, D. R.; Kesten, H. D., and Jobling, J. W.: Effect of Iodine on Cholesterol-Induced Arteriosclerosis, Arch. Path. **20**:337, 1935. (*e*) Turner, K. B., and Bidwell, E. H.: Some Effects of Iodine in Rabbits After Cholesterol Feeding, Proc. Soc. Exper. Biol. & Med. **35**:656, 1937.

3. Duff and McMillan.[2b] Kellner and others.[2c] Meeker and others.[2d] Turner and Bidwell.[2e]

before any further treatment was undertaken, in order that the state of hyperlipemia induced by cholesterol feeding might subside.

Thirty-six cholesterol-fed animals were divided into three equal groups, comprising a control series, a group treated with thyroxin, and a group treated with dinitrophenol. The surviving animals were killed and autopsies made after 13 weeks of treatment. The aortas were carefully examined and graded with reference to the severity of the atherosclerosis present on a scale of 0 to 4.[3b] All tissues and organs were examined microscopically.

Thyroxin (British Drug Houses) was injected subcutaneously as a 0.05% solution in N/50 NaOH. The dose was 1.5 mg., given once a week. 2,4-Dinitrophenol was injected subcutaneously twice daily five days each week in a maximum concentration of 2% in 2% aqueous sodium bicarbonate. Treatment began with a dose of 50 mg. of dinitrophenol, given twice

Summary of Experimental Data

	90-Day Period of Cholesterol Feeding		91-Day Period of Retrogression and Treatment			
Rabbit	Cholesterol Eaten, Gm.	Av. Serum Total Cholesterol, Mg./100 Cc.	Av. Serum Total Cholesterol, Mg./100 Cc.	Change of Body Weight, %	Atherosclerosis of Aorta (Grades 0-4)	Treatment
L 91	67	370	29	— 9.0	0	None (control animal)
M 7	67	530	21	± 0.0	0	None (control animal)
M 1	67	280	37	+ 4.4	1	None (control animal)
M 11	61	527	33	+ 10.0	1	None (control animal)
M 13	66	648	42	+ 3.0	2	None (control animal)
M 2	62	842	103	+ 5.0	2	None (control animal)
L 96	66	1,017	71	+ 18.5	2	None (control animal)
L 94	67	581	42	+ 3.0	3	None (control animal)
M 10	67	625	68	+ 2.9	3	None (control animal)
M 14	67	1,264	190	± 0.0	3	None (control animal)
M 6	64	529	50	+ 2.2	4	None (control animal)
Av. values	65.5	666.5	62.3	.....	..	
M 80	62	143	20	— 8.5	0	19.5 mg. thyroxin
M 79	63	281	30	— 17.0	0	19.5 mg. thyroxin
M 77	62	613	28	— 10.0	0	19.5 mg. thyroxin
M 71	62	233	48	— 6.0	1	19.5 mg. thyroxin
M 75	58	643	50	— 8.1	1	19.5 mg. thyroxin
M 83	60	968	71	— 5.6	1	19.5 mg. thyroxin
M 73	63	730	122	— 4.2	3	19.5 mg. thyroxin
M 72	61	836	56	— 9.2	3	19.5 mg. thyroxin
M 82	63	943	129	— 5.0	4	19.5 mg. thyroxin
M 74	58	989	56	— 6.0	4	19.5 mg. thyroxin
M 78	63	1,490	84	— 20.0	4	19.5 mg. thyroxin
Av. values	60.9	724.5	64.0	.....	..	
L 98	64	331	17	± 0.0	1	198 mg. dinitrophenol daily average
L 97	66	533	36	— 4.3	2	198 mg. dinitrophenol daily average
L 96	67	595	64	— 3.4	2	198 mg. dinitrophenol daily average
L 85	67	627	87	— 2.5	4	190 mg. dinitrophenol daily average
Av. values	66.0	521.5	51.5	.....	..	

daily. The dose was gradually increased as tolerance developed, until the animals were receiving 240 mg. daily after six weeks of treatment. This dose was reduced to 200 mg. daily from the 9th to the 13th week.

Samples of blood for chemical analysis were taken from all animals every three weeks during the initial phase of the experiment, and they were taken at weekly intervals during the regressional period. Estimations of the free and the total cholesterol content of the serum, of the lipid phosphorus, and of the fatty acids of neutral fat were made on all samples.

OBSERVATIONS

Eleven of the control animals and 11 of the thyroxin-treated animals survived the experiment. Only four of the dinitrophenol-treated rabbits survived, the remaining eight having died of acute hyperpyrexia induced by the drug.

The animals treated with thyroxin were hyperreactive, had warm, flushed ears and an increased respiratory rate, and showed increased thirst and appetite. All of these animals lost weight, although they ate more food than the control animals. The latter gained a small amount of weight. At autopsy some of the animals revealed involution of the thyroid gland, hyperplasia of the bone marrow, hyperplasia of the lymphoid elements of the thymus and the spleen, and lymphocytic infiltration of the periportal regions of the liver.

The rabbits treated with dinitrophenol manifested severe transient hyperventilation and hyperpyrexia after each injection. The dose was kept at the upper limit of toleration. Eight of the 12 animals in this group died from acutely toxic overdoses of the drug. The surviving animals lost weight, although they ate more food than the control animals. Like the animals treated with thyroxin, some of those receiving dinitrophenol had involution of the thyroid gland and hyperplasia of the bone marrow.

The control rabbits showed the usual atherosclerotic lesions and changes associated with cholesterol feeding but were otherwise normal.

The experimental data relating to the cholesterol content of the serum of the animals during the course of the experiment, together with the estimation of severity of the aortic atherosclerosis present at autopsy, are shown in the table.

Neither the thyroxin nor the dinitrophenol treatment appeared to have affected the quantities or the relationships of the serum lipids. The degree and the character of the atherosclerotic involvement of the aorta, as judged by macroscopic and microscopic examination, were about the same in each of the three groups of animals. It was observed, however, that the aortic lesions of the animals treated with thyroxin were somewhat more fibrotic and had a slightly greater tendency toward atheromatous necrosis at their centers than those of the control animals.

COMMENT

It is possible to inhibit partially or completely the development of experimental cholesterol atherosclerosis by various means. Some agents, probably including thyroxin, appear to act by interfering with the development of hypercholesteremia.[4] Other agents, such as sorethytan (20) monooleate (tween 80® [5]), and the diabetic state induced by alloxan [6] act by altering the physicochemical state of the lipids of the blood and inhibit the development of the lesions in spite of the occurrence of hypercholesteremia.

According to Anitschkow,[1] the well-developed aortic lesions of experimental cholesterol atherosclerosis undergo spontaneous retrogression very, very slowly. Their rate of retrogression is not appreciably influenced by the alloxan diabetic

4. Turner, K. B.: Studies on the Prevention of Cholesterol Atherosclerosis in Rabbits: I. The Effects of Whole Thyroid and of Potassium Iodide, J. Exper. Med. **58**:115, 1933. Breusch, F., and Thiersch, H.: Der Einfluss des Jods auf die Kaninchenatheromatose, Ztschr. ges. exper. Med. **95**:458, 1935.

5. Payne, T. P. B., and Duff, G. L.: The Effect of Tween 80® on the Serum Lipids and the Tissues of Cholesterol-Fed Rabbits, A. M. A. Arch. Path. **51**:379, 1951. Kellner and others.[2c]

6. Duff, G. L., and Payne, T. P. B.: The Effect of Alloxan Diabetes on Experimental Cholesterol Atherosclerosis in the Rabbit: III. The Mechanism of the Inhibition of Experimental Cholesterol Atherosclerosis in Alloxan Diabetic Rabbits, J. Exper. Med. **92**:299, 1950. Duff and McMillan.[2b]

state,[2b] nor by the use of the detergent sorethytan (20) monooleate,[2c] although both these induce profound disturbances in lipid metabolism. The lipotropic agent choline is without effect on the retrogression of the lesions.[2a]

SUMMARY

The present experiment has demonstrated that thyroxin and 2,4-dinitrophenol, two agents that greatly increase cellular metabolism, fail to hasten the retrogression of well-developed atherosclerotic lesions in rabbits. The drugs were given in doses that resulted in physiological and morphological evidence of activity. It may be noted also that Stamler and associates [7] failed to influence the development of experimental atherosclerotic lesions in chickens by the use of nontoxic doses of dinitrophenol.

7. Stamler, J.; Silber, E. N.; Miller, A. J.; Akman, L.; Bolene, C., and Katz, L. N.: The Effect of Thyroid and of Dinitrophenol-Induced Hypermetabolism on Plasma and Tissue Lipids and Atherosclerosis in the Cholesterol-Fed Chick, J. Lab. & Clin. Med. **35**:351, 1950.

FAMILIAL GINGIVAL HYPERTROPHY IN THE DOG (BOXER BREED)

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AND

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THIS PAPER deals with a study of gingival biopsies of four dogs, all boxers of close ancestry, exhibiting gingival hyperplasia. Microscopically, the lesions were characterized by a central area of bone and by nests and cords of cells suggestive of odontogenic epithelium. A review of the literature failed to reveal a similar study.

METHODS

The biopsy specimens were fixed in 10% formalin, embedded in paraffin, sectioned serially, and stained with hematoxylin and eosin. Specimens containing calcified tissue were decalcified according to the method described by Morse.[1]

REPORT OF CASES

CASE 1.—A 4-year-old male boxer exhibited hypertrophic gingiva. This condition had been present for seven months. Raised areas were present on the buccal and labial mucosa (Fig. 1). A solitary, slightly pedunculated lesion was present in the area of the lower second premolar. The hypertrophic tissue exhibited a whitish translucency with a pink background.

Microscopic Description.—On one surface, the tissue is covered by a stratified squamous epithelium which is parakeratotic. The underlying connective tissue stroma consists of bundles of collagenous fibers. Deep in the specimen is a cyst which is lined by squamous epithelium. The cystic cavity contains a mucin-like material within which are macrophages and cellular debris (Fig. 2). Adjacent to the cystic cavity are cords of epithelial cells, some of which are continuous with the cystic lining (Fig. 3). Several foci of inflammatory cells (plasmacytes and lymphocytes) are visible. To one side of the cyst is mature lamellated bone. At the periphery, new bone is present.

CASE 2.—This boxer was a 7½-year-old male. The duration of the lesion was one year. Gingival hypertrophy was present on the labial surface of the maxilla from premolar to premolar. In the mandible, only the gingiva adjacent to the incisors was involved. Biopsy specimens were removed from the maxillary region adjacent to the right canine and from the mandible.

Microscopic Description.—The specimens of soft tissue are covered on one surface by a parakeratotic stratified squamous epithelium. A mild inflammatory response is present in the lamina propria. Deep in the stroma are cords of epithelial cells which appear to be actively proliferating. Lamellated bone is also present in the stroma.

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Assistant Professor of Pathology, University of Illinois College of Dentistry (Dr. Burstone); Administrator, Department of Animal Hospital, University of Illinois (Dr. Bond).

1. Morse, A.: Formic Acid-Sodium Citrate Decalcification and Butyl Alcohol Dehydration of Teeth and Bones for Sectioning in Paraffin, J. Dent. Res. **24**:143, 1945.

Fig. 1.—Buccal aspect of gingival lesions.

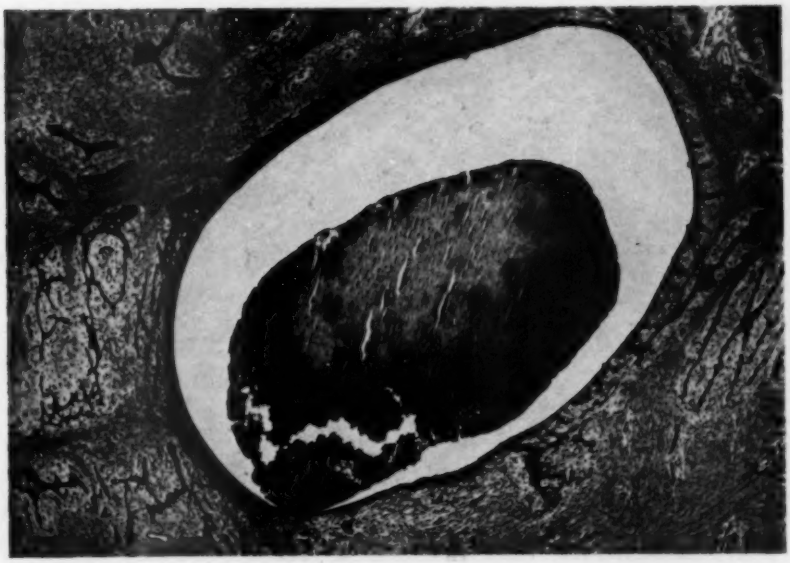

Fig. 2.—Cystic cavity and adjacent strands of epithelium; × 60.

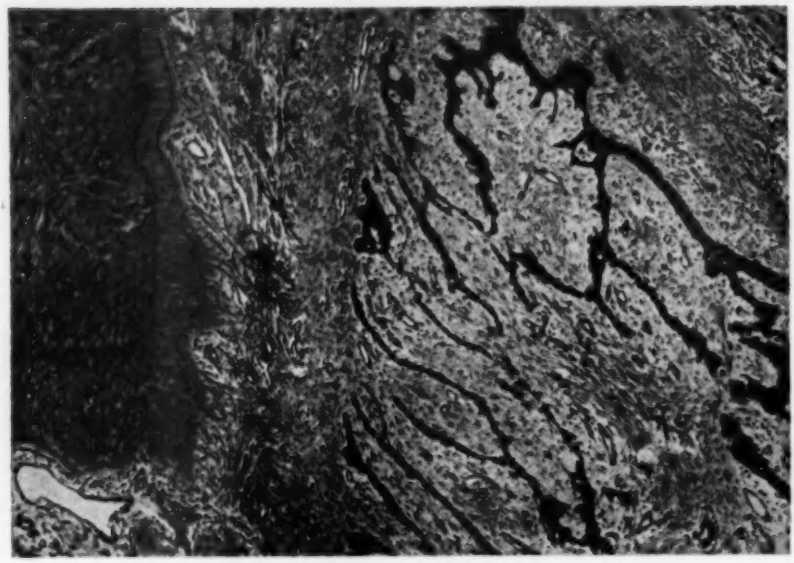

Fig. 3.—Strands of epithelium and lamellated bone; × 100.

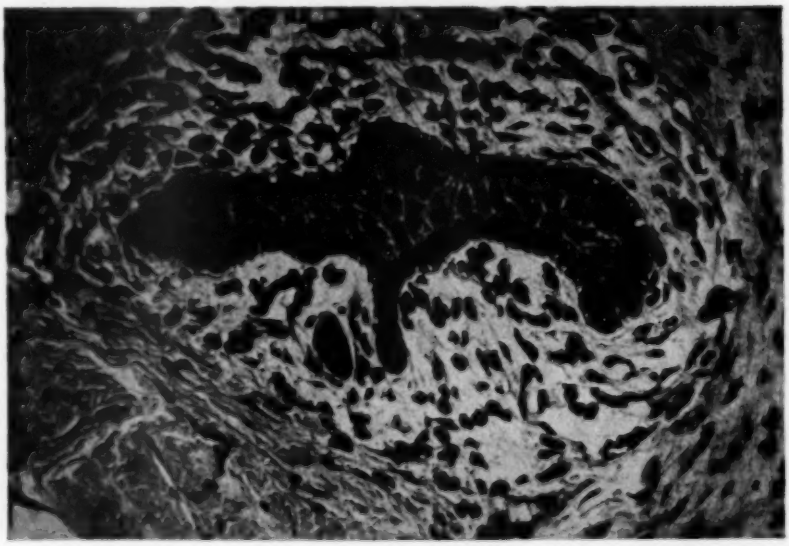

Fig. 4.—High magnification of epithelium and loose surrounding connective tissue; × 600.

CASE 3.—This 8-year-old male boxer exhibited markedly hypertrophic gingiva involving both upper and lower jaws. In some areas most of the coronal part of the tooth was covered by soft tissue. A gingivectomy was performed, and the excised tissue was examined histologically. A recurrence of the hypertrophic tissue was seen six months later but was much less prominent than the original lesion.

Microscopic Description.—The hyperplastic tissue is covered by a parakeratotic stratified squamous epithelium. The central region of some lesions consists of mature lamellated bone, the marrow of which is fatty. In areas adjacent to the bone are small cords of epithelial cells (Fig. 4). Several cords exhibit an outer layer of cuboidal cells. A diffuse plasmacytic infiltration of the lamina propria is present.

CASE 4.—The boxer was a 6-year-old male. In this case the lesion was less prominent and less extensive than in the previous case cited.

Microscopic Description.—Histologically, case 4 is similar to the other cases. Mature lamellated bone is present together with small strands of epithelial cells.

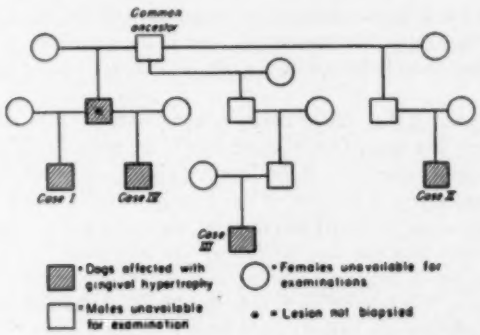

Fig. 5.—Familial relationship of boxers with gingival hypertrophy.

COMMENT

Gingival hyperplasia occurring in dogs has been reported by Mulligan [2] and Riser.[3] However, there is no indication of the etiologic factors or a possible familial relationship of the animals with the gingival lesions. Hereditary gingival fibromatosis occurs in humans, but a review of the literature indicates that the lesions consist of connective tissue covered by epithelium without the presence of bone or cords of epithelial cells in the stroma.[4] Since the boxer lesions may be of odontogenic origin, some mention should be made of odontogenic neoplasms occurring in animals.

Odontogenic tumors comparable to those found in man have been observed in a variety of animals, including cattle and cats. Zegarelli [5] has observed "adamantoblastomas" in mice. He described solid and cystic odontogenic lesions consisting

2. Mulligan, R. M.: Neoplasms of the Dog, Baltimore, Williams & Wilkins Company, 1949, p. 110.

3. Riser, in Canine Surgery, edited by H. P. Hoskins and J. V. LaCroix, Evanston, Ill., North American Veterinarian, Inc., 1949, pp. 192-193.

4. Aimes, A.: Epulides, Austral. J. Dent. **41**:425, 1937.

5. Zegarelli, E. V.: Adamantoblastomas in the Slye Stock of Mice, Am. J. Path. **20**:23, 1944.

of a squamous type of epithelium, and adjacent connective tissue stroma. Orr [6] reported an "adamantinoma" of the jaw occurring in a rabbit. Microscopically, there were cystic areas lined by a stratified squamous epithelium with a basal cell layer consisting of cuboidal-shaped cells. The cystic cavities contained numerous degenerating desquamated cells. Other areas of this "tumor" consisted of cords of epithelial-type cells.

Although many of these lesions have been described as ameloblastoma (adamantinoma), it is questionable whether they represent ameloblastoma in the strict sense of the term. Ameloblastoma or adamantinoma, defined according to the classification approved by the American Academy of Oral Pathology, is a soft tissue tumor derived from oral epithelium that has the power to differentiate into ameloblasts.[7] The various types of "ameloblastomas" which have been described in animals did not consist entirely of cells which were columnar or cuboidal in appearance as in the typical ameloblastoma. The cases here reported did show epithelium which can be interpreted as being of odontogenic origin, but it is doubtful if they, too, represent true ameloblastomas. The presence of bone in the lesions might be explained as a result of the inductive influence of the odontogenic epithelium on the connective tissue.[8]

The boxer breed in the United States is comparatively new and can be traced back to a relatively few dogs. One of these dogs is the grandsire of three of the dogs affected and the great-grandsire of the fourth (Fig. 5). One of his sons who also has gingival hypertrophy is the sire of the dogs in Cases 1 and 4. Unfortunately, the other dogs shown on the chart and their siblings were not available for examination. It is apparent that the familial factors are important in the genesis of the lesion. However, we do not know whether such lesions are peculiar to the boxer, or to a strain of boxer. It is interesting to note that "familial multilocular cystic lesions" of the jaws (of odontogenic origin) have been reported in man.[9]

SUMMARY

Gingival hypertrophy was studied in four male boxers, which had common ancestry. Histologic study of the lesions revealed cords of epithelium, cystic changes in one specimen, and mature lamellated bone. The presence of bone is interpreted as the effect of an inductive influence of the epithelial component. The histologic appearance suggests that these lesions contain tissue of odontogenic origin.

6. Orr, J. W.: Adamantinoma of the Jaw in a Rabbit, J. Path. & Bact. **42**:703, 1936.

7. Thoma, K. H.: Oral Pathology, St. Louis, C. V. Mosby Company, 1950.

8. Oral Histology and Embryology, edited by B. Orban, St. Louis, C. V. Mosby Company, 1944.

9. Jones, W. A.: Familial Multilocular Cystic Disease of the Jaws, Am. J. Cancer **17**:1946, 1933.

Case Reports

CONGENITAL GOITER WITH DEATH FROM MILK-ASPIRATION PNEUMONIA

Report of a Case with Autopsy

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IN NORTH AMERICA congenital goiter is a rare, usually nonfatal condition. A review of the literature by Jones [1] in 1951 revealed that only six cases in which autopsy was performed had been reported from North America. Jones added two cases and in a footnote stated that an additional case had been reported by Seligman and Pescovitz [2] since his manuscript was submitted for publication. The reported clinical and autopsy incidence of this disease is considerably higher in Europe than in North America.

Potter,[3] in discussing the thyroid gland as observed at birth, stated that there may be considerable variation in weight in different communities. She lists the weights of the thyroid glands of mature newborn infants of the Chicago area as ranging from 1.5 to 3.0 gm. She cited an unpublished series of Dr. Fred Adair, Minneapolis, where many weights of 5 to 7 gm. were recorded. Two of the thyroid glands in Dr. Adair's series were unusually large, one weighing 42 gm. and one 14 gm. Both of these showed microscopic hyperplasia. A number of reports in both the European and the American literature dealing with congenital goiter stress the observation that it occurs in infants born of mothers with iodine deficiency, especially those with goiter. There are instances of its having occurred also in infants whose mothers were receiving thiouracil therapy.

In the infant whose case is to be reported, death occurred from milk-aspiration pneumonia, a complication of congenital goiter not previously recorded in this country. This infant, born prematurely at seven months, also presented an interventricular septal defect.

REPORT OF CASE

Clinical Course.—The mother was a 32-year-old Jewish woman with a history of myxedema of several years' duration. Her first two pregnancies had resulted in abortions at seven months and four months, respectively. In her third pregnancy she gave birth to a normal female infant, who was 7 years of age when the baby who is the subject of the present report was born. Following that birth she had two early abortions and one ectopic pregnancy before the present baby was conceived. The basal metabolic rate over the past several years has ranged from 0

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1. Jones, D. B.: Congenital Goiter in North America, Am. J. Path. **27**:85 (Jan.-Feb.) 1951.

2. Seligman, B., and Pescovitz, H.: Suffocative Goiter in Newborn Infant, New York J. Med. **50**:1845 (Aug. 1) 1950.

3. Potter, E. L.: Pathology of the Fetus and the Newborn, Chicago, Year Book Publishers, Inc., 1952.

to —34. She was kept on desiccated thyroid therapy during the last pregnancy but she could tolerate only 1.5 to 2 grains (0.097 to 0.130 gm.) of thyroid daily. Much of the time she was able to take only 1 grain (0.065 gm.) daily. She had gained 48 lb. (21.7 kg.) during the pregnancy. She had noted marked ankle edema for several weeks before delivery. Except for the edema of the ankles, physical examination at the time of her admission in labor showed no abnormalities. The thyroid gland was not palpable.

The baby was a boy, born prematurely at seven months, weighing 3 lb., 10 oz. (1,644 gm.). Delivery was spontaneous and uneventful. Slight cyanosis was noted in the baby at birth, and physical examination revealed bilateral swelling of the neck in the region of the thyroid gland. Oxygen was administered, and the baby's condition improved temporarily.

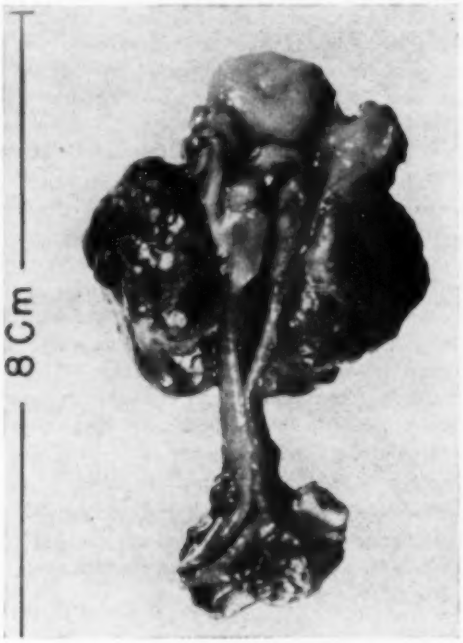

Fig. 1.—Posterior view of the enlarged thyroid gland pressing on the larynx and on the trachea. The esophagus has been removed and the lobes of the thyroid are forced apart to show larynx and trachea.

Intermittent slight cyanosis continued, and on the third day after delivery a loud systolic apical murmur was heard. The baby improved for several days, but on the ninth day after delivery cyanosis and dyspnea became prominent, and numerous fine rales were heard throughout both lung fields. The cyanosis became more marked, and the rales became loud and crackling, and the baby died on the 14th day after birth. The temperature did not rise above 99.6 F. except on the 10th day, when it rose sharply to 103 F. and then in a few hours dropped to 98 F. Treatment included administration of oxygen, penicillin, thyroid, strong iodine solution U. S. P., and intravenous and subcutaneous fluids. The baby was given half-strength breast milk beginning on the fifth day and full-strength breast milk beginning on the 11th day. The baby was difficult to feed. The clinical diagnoses were congenital goiter and congenital heart disease.

Autopsy (performed five hours after death).—There were three important findings: enlargement of the thyroid gland, aspiration pneumonia, and an interventricular septal defect. The baby weighed only 3 lb.

All of the neck organs, including the larynx and the base of the tongue, were removed en masse. An enlarged thyroid gland, measuring 5.0 by 3.5 by 3.0 cm., occupied most of the space in the neck and completely surrounded the trachea and the esophagus. The mass encircled both larynx and esophagus, and it compressed both, although the two lobes did not quite touch posteriorly. Compression of the larynx and the upper portion of the esophagus by the

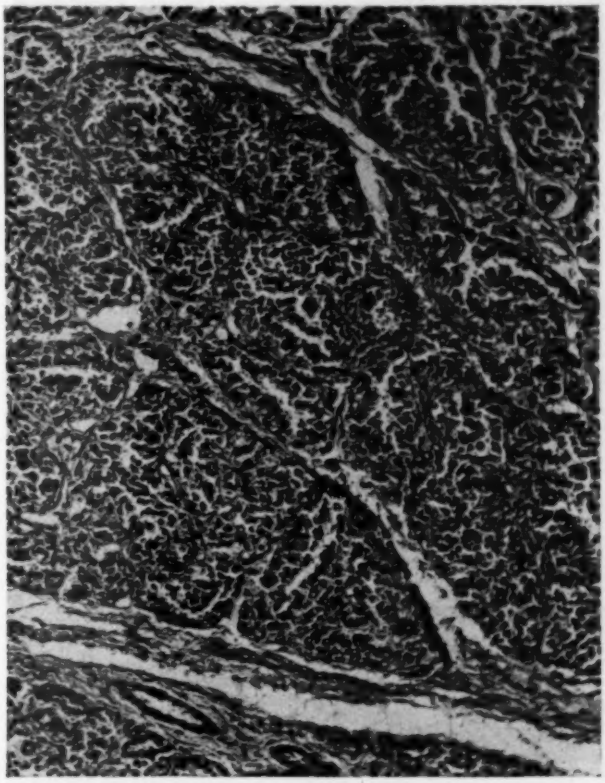

Fig. 2.—The hyperplastic thyroid gland has a solid appearance caused by infolding of the glands and filling of the lumens with desquamated cells. Hematoxylin and eosin; × 150.

mass had converted the openings of these passages into a common pocket, from which, obviously, any ingested material could easily enter the larynx.

The thyroid gland weighed 12 gm. Its outer surface was firm, gray, slightly nodular, and irregular. The capsule was thin. The cut surface was uniformly flat, smooth, glistening, and gray. The thymus gland, weighing 2.5 gm., consisted of a thin strand of fat and fibrous tissue containing thymic remnants.

The left lung weighed 20 gm.; the right, 26 gm. Both lungs showed extensive hemorrhagic pneumonia involving all lobes. The lower lobes were entirely solid, removed portions dropping immediately to the bottom of the fixing solution. The bronchi were markedly injected. The

heart weighed 18 gm. An ovoid defect of the intraventricular septum, measuring 0.8 cm. in greatest diameter, was found just beneath the aortic valve. No other gross abnormalities were noted.

Microscopic examination of the thyroid showed conspicuous diffuse hyperplasia with papillary infolding of many of the glands to such an extent that the lumens appeared obliterated. Desquamated round or polyhedral cells filled many of the remaining luminal spaces, giving these glands a solid appearance. The glands were lined by cuboidal or occasionally columnar

Fig. 3.—Milk in a bronchiole. A pneumonic reaction is seen around the bronchiole. Hematoxylin and eosin; × 150.

epithelium. Occasional glands were partially or completely filled with pale colloid, but the vast majority were empty or filled only with desquamated cells. Occasional fine strands of fibrous tissue divided some areas into lobules.

Microscopically, the lungs showed extensive hemorrhagic aspiration pneumonia. Aspirated milk was seen as amorphous hyaline-like material both in the bronchi and in the alveoli. This material contained tiny pink or red granules, seen only under high-power magnification. The tissue response included both an acute pneumonia and a chronic granulomatous reaction. Part of the reaction was caused by the protein component of the milk and part by the fat.

In the bronchi the aspirated material was surrounded by numerous red blood cells and by a few segmented neutrophiles. Numerous red blood cells, segmented neutrophiles, and monocytic phagocytes were found in alveolar spaces. The phagocytic cells were large, pale, foamy, and finely vacuolated, somewhat resembling the lipophages seen in bronchopneumonia due to aspiration of oil (lipid pneumonia). The vacuoles were not so sharply defined, however, as those of lipid pneumonia. Many fine pink or red granules, resembling those seen in the large masses of milk, were seen in the monocytes. In occasional areas the appearance of clear spaces surrounded by monocytes and lymphocytes with early foreign-body giant-cell reaction and

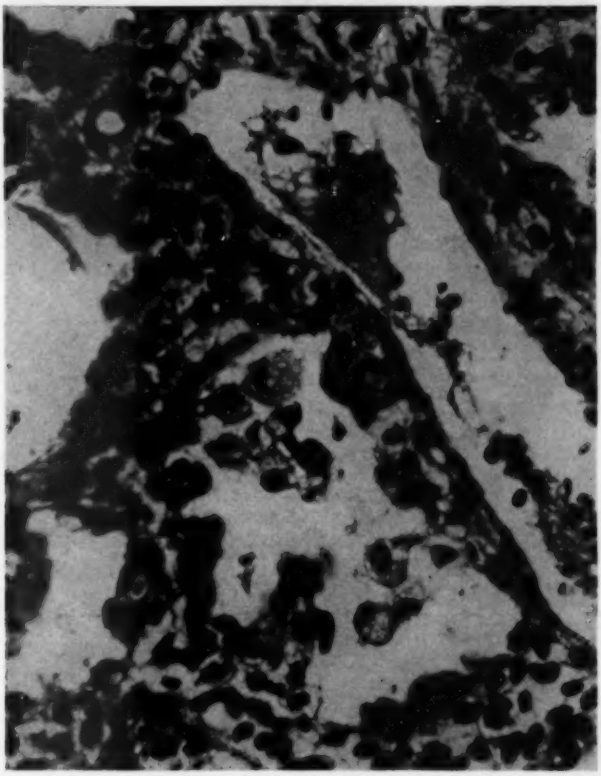

Fig. 4.—Early granulomatous reaction around large clear spaces containing milk and foamy phagocytes. The milk is seen in the upper space surrounded by phagocytes and red blood cells. Hematoxylin and eosin; × 1,000.

deposition of fibroblasts suggested that milk had been present but had been removed during the process of preparing the sections. Frozen sections of the involved portions of lung stained with hematoxylin and eosin and Sudan IV confirmed this impression, as acellular masses of material resembling the fibrinous portion of a thrombus were seen in these spaces. Occasional bits of fat stained with Sudan IV were scattered through these amorphous masses, but most of the fat was seen in monocytic cells around the material. Many monocytes through all of the sections contained small fat globules which stained pink or red with Sudan IV. Extra-

cellular fat globules were also seen. In many areas the inflammatory reaction was interstitial as well as alveolar.

COMMENT

This congenital goiter of hyperplastic type occurring in a newborn, premature infant is of special interest because of the mother's history of long-standing myxedema and of repeated abortions. The enlargement of the thyroid gland (12 gm.) is relatively great when the baby's weight of 3 lb. is considered. Any attempt to relate

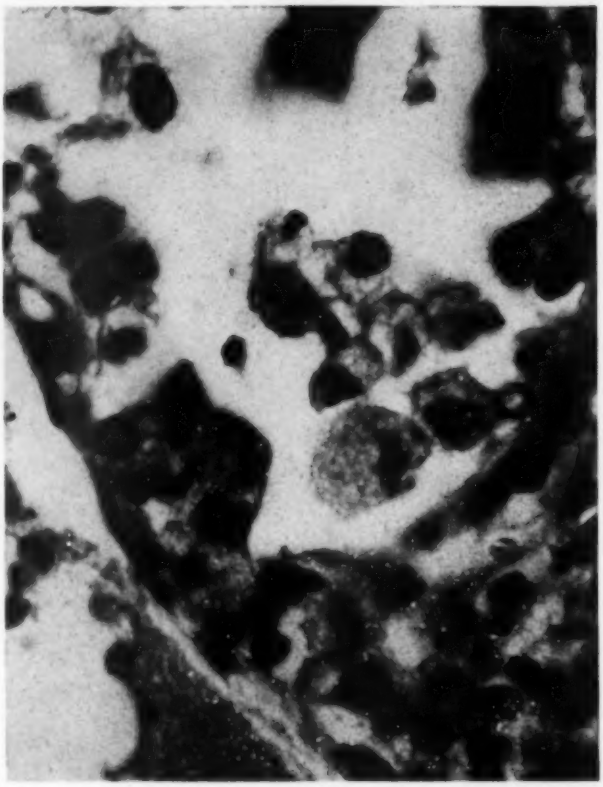

Fig. 5.—Phagocytic cells with vacuolation due to milk fat. An early granuloma is seen around the phagocytes. Hematoxylin and eosin; × 1,800.

the mother's thyroid deficiency to the infant's interventricular septal defect would be purely speculative. The wasting of the thymus gland is interesting, but it is probably not related to the thyroid hyperplasia, as it may occur with a variety of diseases.

Although milk-aspiration pneumonia has not previously been described in the North American literature as a cause of death in newborn infants with goiter, it is not a surprising complication of congenital goiter in those infants who live long

enough to be fed. Any enlargement of the thyroid gland of sufficient bulk to press on the larynx and the esophagus obviously exerts greater compression on the soft elastic esophagus than on the rigid larynx and consequently favors laryngeal aspiration of ingested material.

The milk may be difficult to identify in histologic sections, and much of it, especially the fat, may be removed from the tissues during the preparation of routine paraffin sections. Careful study may be required to differentiate milk from blood fibrin. Knowledge of the appearance of the milk stained by hematoxylin-and-eosin methods and the reaction of the lung to the foreign material make the differentiation possible. Milk-aspiration pneumonia is ordinarily regarded as a variation of lipid pneumonia, but in this instance, as well as in others in my experience, the reaction is due to the protein in the milk as well as to the fat. Inasmuch as both protein and fat are responsible for the condition, the term "milk-aspiration pneumonia" is preferable to "lipid pneumonia caused by milk."

SUMMARY

A case of congenital goiter with marked microscopic hyperplasia is recorded. The baby also presented an interventricular septal defect. The infant's mother had a history of long-standing thyroid deficiency and of repeated abortions.

Death resulted from an extensive milk-aspiration pneumonia presumably caused by the enlarged thyroid pressing on the esophagus. The pathologic features of this condition include a response varying from alveolar and interstitial pneumonia to a chronic granulomatous reaction with fibrosis. The changes are caused by the protein component of the milk as well as by the fat, and the condition is more accurately described by the term "milk-aspiration pneumonia" than by terms such as "lipid pneumonia," which suggest that the response is entirely to fat.

In those infants with congenital enlargement of the thyroid or other masses of the neck which may press on the esophagus, the physician must emphasize to those responsible for the nursing care of the baby the importance of preventing milk or other material from being aspirated into the larynx.

Dr. Ralph E. Tafel, of the Department of Obstetrics and Gynecology, St. Margaret Memorial Hospital, supplied the mother's history.

General Review

STAINING OF CONNECTIVE TISSUE

R. D. LILLIE, M.D.
BETHESDA, MD.

COLLAGEN, RETICULUM, AND BASEMENT MEMBRANES

AS THE study of the structure of animal tissues in their normal state and in various pathologic conditions has progressed, more and more details of the complex structure of tissues and organs have become evident. Collagen, first identified as fibrillar material which swelled in acetic acid and hydrolyzed to form gelatin, was one of the earliest tissue elements to be specifically stained, and this specific staining has aided greatly in an understanding of the processes of scarring, desmoplasia, and fibrosis, as well as of the structure of organs.

Evidence of the widespread interest in the manner in which fibrous stroma is related to tissue and organ structure is furnished by the multiplicity of methods which have been devised for its differential staining. These have varied greatly in specificity and completeness of staining as well as in apparent basic principle.

Historical Review

The Picric Acid Methods.—Apparently the oldest differential connective tissue stain is picroindigocarmine. With this stain Jullien,[1] in 1872, obtained blue connective tissue and yellow parenchymal cells. He used a mixture of a saturated solution of indigocarmine in water acidulated with oxalic acid and concentrated picric acid (trinitrophenol) solution in such proportion as to give a beautiful green color to the mixture.

This method has been attributed to S. Ramón y Cajal[2] by Lee,[3] Langeron,[4] and Masson.[5] He apparently first used it about 1895. His triple stain consisted of a nuclear stain with carmine or, later, a saturated solution of basic fuchsin or Ziehl's carbolfuchsin, followed by washing and 5 to 10 minutes' staining in 0.25% indigocarmine in saturated aqueous picric acid solution. Sections were then rinsed in very weak acetic acid solution and differentiated in absolute alcohol.

From the Laboratory of Pathology and Pharmacology of the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Federal Security Agency.

1. Jullien, L.: Sur une nouvelle méthode de coloration des éléments histologiques, Lyon méd. **10**:526-530, 1872.

2. Ramón y Cajal, S.: Métodos de coloración de las neoplasias, Rev. cien. med. Barcelona **22**:97, 1896; Histology, Translated by M. Fernan-Nunez, Baltimore, William Wood & Company, 1933.

3. Lee, A. B.: Microtomists' Vade Mecum, Philadelphia, The Blakiston Company, Ed. 8, 1924; Ed. 9, 1928; Ed. 10, 1937.

4. Langeron, M.: Précis de microscopie, Ed. 7, Paris, Masson et Cie, 1949.

5. Masson, P.: Some Histological Methods: Trichrome Stainings and Their Preliminary Technique, J. Tech. Methods **12**:75-90, 1929.

The method has since been used by a number of other workers, but it appears to be less selective than a number of other dyes used in picric acid mixtures.

In 1885 Martinotti [6] reported using an alcohol solution saturated with both nigrosin and picric acid for central nervous system tissues. After this stain he differentiated with a 1 : 2 dilution of formic acid in alcohol. Vessel walls were blue, connective tissue and glia fibers lighter blue, myelin yellow, and nuclei pale blue.

Freeborn's [7] picronigrosin stained connective tissue blue, nuclei gray, and other structures yellow. He mixed one part 1% aqueous nigrosin with 9 parts saturated aqueous picric acid. The dye used was designated as nigrosin, indulin, or anilin blue-black and was obtained from Grübler.

In 1889 Van Gieson [8] first reported the use of acid fuchsin-picric acid mixtures. The original directions called for a few drops of saturated aqueous acid fuchsin solution added to saturated aqueous picric acid solution to produce a "dark garnet color." Later Berry [9] prescribed 5 parts of 1% acid fuchsin and 100 parts of saturated aqueous picric acid. This is the usual quoted proportion, adopted by Mallory and Wright,[10] Mallory,[11] Scanlan, French, and Holmes,[12] and others.

Various other proportions and mixtures have been recommended by a series of authors from 1898 on. The best proportion for general use appears to be 0.1% acid fuchsin in saturated picric acid solution. The latter proportion was recommended first by Ramón y Cajal, according to Schaffer,[13] who also used it, then by Hansen,[14] in 1899, Weigert,[15] Hornowski,[16] and Lillie.[17] This mixture remains one of the most generally useful of the connective tissue stains. Besides collagen, it stains bone matrix and osteoid tissue vividly and colors gelatin deep red. Ocular lens capsule and Descemet's membrane are stained by it.

Dubreuil [18] in 1904 reported using picric acid mixtures with certain "acid methyl blues" to obtain specific blue connective tissue staining. He added 4 cc. of

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6. Martinotti, G.: La picronigrossina nella studio delle alterazioni dei centri nervosi, Ztschr. wissensch. Mikr. **2**:478-494, 1885.

7. Freeborn, G. C.: Notes on Histological Technique, Am. Month. Microscop. J. **9**:231, 1888.

8. Van Gieson, I.: Laboratory Notes of Technical Methods for the Nervous System, New York M. J. **50**:57-60, 1889.

9. Berry, J. M.: A Comparison of the Phagocytic Action of Leucocytes in Amphibia and Mammals, Tr. Am. Microscop. Soc. **19**:95-105, 1898. Lee attributes this to Van Gieson.

10. Mallory, F. B., and Wright, J. H.: Pathological Technique, Ed. 8, Philadelphia, W. B. Saunders Company, 1924.

11. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938.

12. Scanlan, J. F.; French, R. W., and Holmes, W. C.: Acid Fuchsin as a Stain—a Refinement in the Manufacture, Stain Technol. **2**:50-55, 1927.

13. Schaffer, J.: Zur Kenntnis der glatten Muskelzellen, insbesondere ihrer Verbindung, Ztschr. wissensch. Zool. **66**:214-268, 1899.

14. Hansen, C. C.: Eine zurverlässige Bindegewebsfärbung, Anat. anz. **15**:151-153, 1899.

15. Weigert, K.: Eine kleine Verbesserung der Hämatoxylin-van Gieson-Methode, Ztschr. wissensch. Mikr. **21**:1-5, 1904.

16. Hornowski: Gleichzeitige differenzielle Färbungsmethode des Bindegewebes, der Muskelfasern und der elastischen Fasern, Przegl. lekarski No. 44, 1908; Ztschr. wissensch. Mikr. **26**:128-192, 1909.

17. Lillie, R. D.: Acid Fuchsin as a Connective Tissue Stain After Phosphomolybdotungstic Mordanting, Stain Technol. **15**:159-165, 1940.

18. Dubreuil: Le picro-bleu; note sur l'emploi de ce réactif pour la coloration spécifique des fibrilles conjonctives, Compt. rend. l'assoc. anat. Toulouse **6**:62, 1904.

0.5% "methyl blue" to 46 cc. of saturated aqueous picric acid. This is a concentration of 40 mg. per 100 cc., which is well within the range of effective action as I have observed it.

Curtis [19] in 1905 described extended studies of picric acid mixtures with various acid dyes, as connective tissue stains, both those of the triphenylmethane group and those of the azo group. In both groups the more highly sulfonated dyes were superior, and in the first group the position of the sulfonic acid groups seemed to be important. While acid fuchsin (C. I. No. 692), red violet 5 RS (C. I. No. 693), and red violet 4 RS (C. I. 694) all gave good staining and exhibited nuclear sulfonation only, he stated that all the di- and tri-sulfonated triphenylmethanes which were sulfonated partly or entirely in the side chains failed to give elective connective tissue staining. These included carmine blue (C. I. No. 712 or patent blue V), Bavarian blue, and Helvetia blue (both are C. I. No. 706 or methyl blue), water-soluble anilin blue (C. I. No. 707), acid green J (C. I. No. 670 or light green SF), formyl violet S4B (C. I. No. 698), violet 6B (C. I. No. 697), violet 10B (C. I. No. 696 or fast acid violet 10B), and solid green extra bluish (probably C. I. No. 671 or erioglaucine; this synonym is not identifiable, but C. I. No. 671 corresponds in color and chemical description).

I am not able to understand some of these results, as anilin blue and methyl blue have given others excellent results as connective tissue stains from picric acid mixtures. I have used several of the above dyes, and while some have performed poorly, others, notably methyl blue, anilin blue, erioglaucine, and light green, have done well. Curtis' theory about the inimical effect of side chain sulfonation does not seem tenable.

Curtis further employed 17 azo dyes in picric acid mixtures. The four best were naphthol black B (C. I. No. 315), diamine blue 2B (C. I. No. 406 or benzo blue BB), diamine blue 3B (C. I. No. 477 or trypan blue), and diamine blue pure A (possibly C. I. No. 520 or benzo sky blue; Conn prefers the name Niagara blue 4B). Benzo blue BB and trypan blue gave me fairly good results, but were not outstanding. Next in order of efficiency, according to Curtis, were ponceau 6R (C. I. No. 186) and ponceau S extra (C. I. No. 282). These he considered equal to acid fuchsin. After these he ranked special ponceau St. Denis (C. I. No. 185 or cochineal red A), ponceau 5R (C. I. No. 254 or erythrin), and an unidentified azo dye with three sodium sulfonate groups called solid red D (possibly C. I. No. 184 or amaranth, which was sold as fast red D by the Badische Anilin u. Sodafabrik), then diamine black BH (C. I. No. 401), diamine blue BX (C. I. No. 472 or benzo blue BX), azofuchsin G (C. I. No. 153), and azorubin S (C. I. No. 179 or azorubine). Of this group, I have tried only azofuchsin G and obtained fair results.

Indifferent results were obtained with a ponceau of Hoechst's which I cannot identify, Bordeaux R (C. I. No. 88), ponceau 4R (C. I. No. 80 is ponceau 3R; this dye was called 4R by the Aktienzgesellschaft für Anilin-Fabrikation, a firm which furnished Curtis with some of his dyes; the other dye, C. I. No. 79, for which ponceau 4R is a synonym was so called by an English firm only), and Biebrich scarlet (C. I. No. 280). My results with Bordeaux R, ponceau R (C. I.

19. Curtis, F.: Nos méthodes de coloration élective du tissu conjonctif, Arch. méd. expér. et anat. path. **17**:603-636, 1905.

No. 79), and Biebrich scarlet were as least as good as those I obtained with benzo blue BB and trypan blue, two of Curtis' best, and I am forced to dissent from Curtis' theory of the superiority of tri- and tetra-sulfonic acid dyes.

Curtis made the significant observation that Victoria yellow (C. I. No. 8), which is the potassium salts of dinitroortho- and para-cresol, naphthol yellow (C. I. No. 10), which is potassium 2,4-dinitro-a-naphthol-7-sulfonate, aurantia (C. I. No. 12), which is the ammonium salt of hexanitrodiphenylamine, dinitrocresol, dinitronaphthol, and dinitroresorcin could not take the place of picric acid in these mixtures. Trinitrocresol, trinitronaphthol, and trinitroresorcin could be substituted for picric acid. Victoria yellow and Martius yellow both yield precipitates of color acid on addition of hydrochloric acid. It is probable that the trinitro compounds are all stronger acids than the dinitro homologues.

By increasing the proportion of acid fuchsin in the Van Gieson mixture, Ohmori (1921)[20] was often able to stain the membrana propria of the renal glomerulus. However, a deep blue-green mixture of saturated aqueous picric acid and saturated methyl blue (C. I. No. 706) was even more efficient for this purpose.

Variants of the last mixture I have used extensively, both in simple combination with nuclear hematoxylin stains and in more complicated procedures designed to give contrasting colors to other tissue elements.

Phosphomolybdic and Phosphotungstic Acid Methods.—The second large group of specific connective tissue stains is that which utilizes phosphomolybdic or phosphotungstic acid before or with the collagen stain. The original method reported by Mallory[21] in 1900 gave a sequence of one to three minutes in a plasma stain of 0.05 to 0.1% acid fuchsin, one minute in a mordant of 1% phosphomolybdic acid, two washes in water, and 2 to 20 minutes in the fiber and plasma stain mixture of 0.5% water-soluble anilin blue (C. I. No. 707), 2% orange G, and 2% oxalic acid. He stated that fixatives containing mercuric chloride, preferably Zenker's fluid, were required.

In 1905 Mallory[22] combined the mordant and the fiber stain, omitted the oxalic acid, and changed the concentration of the acid fuchsin to 0.5%. After five minutes in the 0.5% acid fuchsin, tissues were stained for 10 to 20 minutes in the mixture of mordant and counterstain. In 1936 he[23] substituted phosphotungstic for phosphomolybdic acid and recommended staining for 30 minutes in 0.25% acid fuchsin followed by direct transfer to a mixture of 0.5% anilin blue, 2% orange G, and 1% phosphotungstic acid for 1 to 24 hours or one hour at 56 C.

Woolley[24] in 1903 used Mallory's first method with maximum staining times and 0.1% acid fuchsin and introduced a further differentiation in anilin. Bartel and Stein[25] (1905) followed Woolley's method, increasing the mordant time to

20. Ohmori, D.: Bedeutung der Membrana propria de Glomerulusschlingen in der Nierenpathologie, Arch. path. Anat. **23**:53-72, 1921.

21. Mallory, F. B.: A Contribution to Staining Methods, J. Exper. Med. **5**:15-20, 1900.

22. Mallory, F. B.: A Contribution to the Classification of Tumors, J. M. Res. **13**:113-136, 1905.

23. Mallory, F. B.: The Anilin Blue Collagen Stain, Stain Technol. **11**:101-102, 1936.

24. Woolley, P. G.: A Study of the Reticular Supporting Network in Malignant Neoplasms as Stained by Mallory's Method, Bull. Johns Hopkins Hosp. **14**:21-24, 1903.

25. Bartel, J., and Stein, R.: Lymphdrüsen und Tuberkulose, Arch. Anat. u. Physiol., Anat. Abt. 141-158, 1905.

five to seven minutes and decreasing the orange G from 2.0 to 0.2%. These figures were adopted in Schmorl's [26] fourth edition (1907).

In 1916 Heidenhain [27] substituted 0.25 to 1% azocarmine B (C. I. No. 829) or a solution of azocarmine G (C. I. No. 828), saturated by heating, for the acid fuchsin plasma stain in the original Mallory procedure. This staining was prolonged to 30 to 60 minutes at 56 C. followed by one to two hours at 37 C. The solutions were "well acidified." Next he mordanted one-half to three hours in 5% phosphotungstic acid, then rinsed and stained one to three hours in the anilin blue and orange G mixture. The last solution contained 8% acetic acid in place of oxalic acid and was diluted with one to two volumes of water before use. Mallory [11] (1938) described this solution as containing 0.5 gm. of anilin blue, 2.0 gm. of orange G, 8 cc. of glacial acetic acid, and 100 cc. of water and directed a 1 : 3 dilution. He also stated that the azocarmine solutions contained 1% acetic acid. This method has been widely used as probably the best method for complete demonstration of all connective tissue fibrils. In practice it is time-consuming, dependent on a number of microscopic differentiations, and, consequently, scarcely a method for other than the more skilled technicians.

Masson's [5] trichrome methods are in part modifications of the original Mallory method using a sequence of a plasma stain, phosphomolybdic acid, and fiber stain, and in part modified Van Gieson methods in which another plasma stain is introduced. The former is generally meant when reference is made to Masson's trichrome stain. For these methods Regaud or Bouin fixation is prescribed, though formalin and alcohol material may be used by premordanting, respectively, with Regaud and Bouin to increase the intensity of the plasma stain.

First stain nuclei with Regaud's iron-hematoxylin and differentiate in alcoholic picric acid (two-thirds saturated). Wash out the picric acid for 15 minutes in running water. (I have found three minutes quite adequate to remove the picric acid.) Apply plasma stain for five minutes. This is dissolved in 1% acetic acid and consists of 1% xylidine ponceau or 1% acid fuchsin or a mixture of two parts of the first and one part of the second. Rinse in distilled water. Mordant five minutes in 1% phosphomolybdic acid and pass directly into the fiber stain for five minutes. This is 2 to 3% anilin blue in 2.5% acetic or 2% light green in 1% acetic acid or saturated aqueous metanil yellow (C. I. No. 138). Tissues stained in anilin blue are returned to the phosphomolybdic acid for five minutes and then rinsed. All are differentiated, metanil yellow by rinsing in distilled water, the others by two minutes in 1% acetic acid. The acid fuchsin plasma stain was used with the metanil yellow, the ponceau, and the mixture with the other two. Curtis' salicylic balsam or natural acid balsam was used for mounting.

This variation of the Mallory method has also been widely used and considerably modified by several writers: Foot,[28] Patay,[29] Larson and Levin,[30] Lillie,[31] Kornhauser,[32] Lendrum and McFarlane,[33] and others.

26. Schmorl, G.: Die pathologisch-histologischen Untersuchungsmethoden. Leipzig, F. C. W. Vogel, Ed. 4, 1907; Ed. 15, 1928.

27. Heidenhain, M.: Über die Mallorysche Bindegewebsfärbung mit Karmin und Azokarmin als Vorfarben, Ztschr. wissensch. Mikr. **32**:361-372, 1916.

28. Foot, N. C.: The Masson Trichrome Staining Methods in Routine Laboratory Use, Stain Technol. **8**:101-110, 1933.

29. Patay, R.: Une nouvelle technique de coloration tricchromique pour l'histologie, Bull. histol. appliq. à la physiol. **11**:408-409, 1934; abstracted, Stain Technol. **10**:69, 1935.

30. Larson, C. P., and Levin, E. J.: A Modification of Masson's Tetrachrome Stain, Arch. Path. **29**:272-273, 1940.

A development from this group of stains is the appearance of a number of single-solution stains: Ladewig's,[34] Shorr's,[35] the widely used and much publicized Papanicolaou [36] variants, Cason's [37] and Gomori's [38] 1950 method. Ladewig's gave both red and blue collagen; Shorr's and Papanicolaou's have been used almost entirely for smear diagnosis, and hence do not enter into present consideration.

The Cason stain [37] is recommended for staining connective tissue in paraffin sections.

Cason dissolves successively in 200 cc. distilled water, 1 gm. of phosphotungstic acid, then 2 gm. of orange G, 1 gm. of anilin blue, and finally 3 gm. of acid fuchsin.

Paraffin sections are deparaffinized and hydrated as usual (mercury being removed with the iodine thiosulfate sequence if necessary), stained five minutes, dehydrated through graded alcohols, cleared in xylene, and mounted in balsam.

Mendelson (1951) modifies the method for celloidin sections as follows:

Hydrate as usual, stain 15 to 20 *seconds* only, rinse quickly in distilled water, decolorize until celloidin is colorless in 3 cc. of anilin, 12 cc. of chloroform, and 85 cc. of 95% alcohol, rinse in 7:1 95% alcohol-chloroform mixture, clear in terpineol-xylene (1:3) and two changes of xylene, and mount.

The usual color contrasts of the Mallory stain are claimed for these variants.

Gomori [38] (1950) finds that one-solution trichrome mixtures can be made from blue or green acid triphenylmethane or diphenylnaphthylmethane dyes as collagen stains, with red sulfonated azo or disazo dyes as plasma stains, acetic acid, and phosphotungstic or phosphomolybdic acid. Phosphotungstic acid tends to intensify the plasma stain; phosphomolybdic acid, the fiber stain; alcohol weakens the plasma stain. Pretreatment with hot Bouin's fluid intensifies staining of muscle and plasma. Water washing extracts plasma stains more than fiber stains, whereas acetic acid rinsing makes the preparation more transparent without altering the color balance. Gomori recommends a mixture of chromotrope 2R (0.6), fast green FCF (0.3), phosphotungstic acid (0.6), glacial acetic acid (1) in water (100). Connective tissue is stained green, muscle and cytoplasm red, nuclei gray blue. Like the other fast-

31. Lillie, R. D.: Some Experiments with the Masson Trichrome Modification of Mallory's Connective Tissue Stain, J. Tech. Methods **18**:75-81, 1938; Further Experiments with the Masson Trichrome Modification of Mallory's Connective Tissue Stain, Stain Technol. **15**:17-22, 1940.

32. Kornhauser, S. I.: A Revised Method for the "Quad" Stain, Stain Technol. **20**:33-35, 1945.

33. Lendrum, A. C., and McFarlane, D.: A Controllable Modification of Mallory's Trichromic Staining Method, J. Path. & Bact. **50**:381-384, 1940.

34. Ladewig, P.: Über eine einfache und vielseitige Bindegewebsfärbung, Ztschr. wissensch. Mikr. **55**:215-217, 1938.

35. Shorr, E.: New Technic for Staining Vaginal Smears: Single Differential Stain, Science **94**:545-546, 1941. Foot, N. C.: Pathology in Surgery, Philadelphia, J. B. Lippincott Company, 1945, p. 11.

36. Papanicolaou, G. N.: Some Improved Methods for Staining Vaginal Smears, J. Lab. & Clin. Med. **26**:1200-1205, 1941; New Procedures for Staining Vaginal Smears, Science **95**:438-439, 1942. Papanicolaou, G. N., and Cooper, W. A.: Cytology of Gastric Fluid in the Diagnosis of Carcinoma of the Stomach, J. Nat. Cancer Inst. **7**:357-360, 1947.

37. Cason, J. E.: A Rapid One-Step Mallory-Heidenhain Stain for Connective Tissue, Stain Technol. **25**:225-226, 1950.

38. Gomori, G.: A Rapid-One-Step Trichrome Stain, Am. J. Clin. Path. **20**:661-664, 1950.

38a. Mendelson, J.: Carson's Polychrome Stain for Celloidin Embedded Material, Stain Technol. **26**:217-218, 1951.

green methods of the Masson type, it gives a rather diffuse and incomplete picture of the more finely fibrillar stroma. Much of the reticulum of the liver and spleen is difficult to discern.

Counterstained Plasma Stain, Picric Acid Methods.—A third group of methods is that in which a separate plasma stain is used and the picric acid of the Van Gieson-type stain is employed only as a mordant. This procedure was introduced by Masson [8] in 1929 but has had comparatively little attention.

Masson stained first with Regaud's iron-hematoxylin, washed, stained five minutes in 2 cc. of saturated aqueous metanil yellow (C. I. No. 138) in 100 cc. of 1% acetic acid, rinsed in water, mordanted five minutes in 3% potassium dichromate, stained 30 to 60 seconds in 1% acid fuchsin in saturated aqueous picric acid, rinsed in distilled water, differentiated five minutes in 1% acetic acid, dehydrated, cleared, and mounted in salicylic balsam. This stained muscle and cytoplasm yellow and collagen red. Foot [28] quoted this method as given above and supplied the percentage of the acetic acid in the metanil yellow, which Masson had omitted.

My [39] Biebrich scarlet-picro-anilin blue method was an adaptation of this principle:

The dilute metanil yellow was replaced by 0.2% Biebrich scarlet in 1% acetic acid. Sections were stained for four minutes and then rinsed; Masson's bichromate bath was omitted, as comparative tests indicated no material difference; the picrofuchsin was replaced by 0.1% anilin blue (C. I. No. 707) in saturated aqueous picric acid, treatment continuing for four to five minutes. Sections were then transferred directly to 1% acetic acid for differentiation. This was reduced to three minutes.

This method gave blue connective tissue, including much reticulum, glomerular stroma, and mucosal basement membranes. Erythrocytes were orange-scarlet, muscle orange-pink, cytoplasm pink to gray, and nuclei gray to black. In practice, over-use of the same solutions allowed some gray-blue staining of cytoplasm and diminished the selectivity for fine connective tissue fibrils.

Subsequent to these reports I [40] made a rather extended survey of this method and also of the other acid anilin dye methods.

Good contrasts were obtained with a plasma stain in red and brown with azofuchsin G and brilliant purpurin R, followed by picric acid mixtures with the disazo dye naphthol blue black (C. I. No. 246); with a fast-green plasma stain and a picroacid fuchsin collagen stain. The last is one of the most contrastful of the picric acid procedures.

Acidification of picric acid, acid fuchsin mixtures was shown to increase their selectivity.

Further exploration of this effect led to the discovery that triphenylmethane sulfonic acid dyes become quite selective collagen stain at low pH levels, regardless of the source of H ion.

This study was the source of the two "hydrochloric acid methods" which I included in my Histopathologic Technic.[41] These used plasma stains with acetic Biebrich scarlet and with phloxin, followed by a fiber stain with methyl blue or

39. Lillie, R. D.: Biebrich Scarlet-Picro-Anilin Blue: A New Differential Connective Tissue and Muscle Stain, Arch. Path. **29**:705, 1940.

40. Lillie, R. D.: Studies on Selective Staining of Collagen with Acid Anilin Dyes, Bull. Internat. A. M. Mus. **25**:1-47, 1945.

41. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

anilin blue, or naphthol blue black, in 0.25 or 0.5% dilution of concentrated hydrochloric acid.

While these hydrochloric acid methods are of considerable theoretical interest, from the point of view of practicality they are inferior to the better picric acid combinations.

Silver Procedures.—After Maresch [42] discovered in 1905 that the Bielschowsky ammoniacal silver methods would demonstrate the reticulum of the liver, he, Hörmann,[43] Herxheimer,[44] Rössle,[45] Kon,[46] Schmidt,[47] Rössle and Yoshida,[48] Russakoff,[49] down to 1909, and many others at later dates, applied this procedure to various normal and pathologic organs and tissues.

It became evident that the reticulum of the solid viscera was more strongly argyrophilic than the collagen of capsules and general tissues. However, the similarity of staining of reticulum and collagen by the acid anilin dye methods, and the apparent conversion of reticulum to collagen in sclerosing processes, remained, and a lively controversy arose as to the identity or nonidentity of reticulum and collagen.

Basement membranes of glands and epithelia also early became the subject of controversy. His, [50] as early as 1865, apparently regarded basement membrane as a condensation of closely felted reticular fibrils. Von Ebner [51] in 1899 considered the basement membrane as a hyaline structure abutting closely on supporting fibrillar reticulum, but Rühle [52] in 1897, Mall [53] in 1891, and others in the presilver days adopted the concept of a closely felted condensation of reticular fibers. The introduction of the silver methods led to the almost complete suppression of the hyaline membrane theory and the dominance of the concept of a condensation of closely felted argyrophilic reticular fibrils against endothelia, epithelia, and gland cells.

42. Maresch, R.: Über Gitterfasern der Leber und die Verwendbarkeit der Methode Bielschofskys zur Darstellung feinster Bindegewebsfibrillen, Zentralbl. allg. Path. u. path. Anat. **16**:641-649, 1905.

43. Hörmann, C.: Über das Bindegewebe der weiblichen Geschlechtsorgane, Arch. Gynaekol. **82**:619-678, 1907.

44. Herxheimer: Zur Pathologie der Gitterfasern der Leber, 1907; cited by Kon [46] and Russakoff.[49]

45. Rössle, R.: Über das Gitterfasergerüst der Leber, Sitzungsb. Gesellsch. Morph. u. Physiol. Munich, 1908; cited by Rössle and Yoshida, 1909; Über die Metaplasie von Gitterfasern bei wahrer Hypertrophie der Leber, Verhandl. deutsche path. Gesellsch. **12**:249-253, 1908.

46. Kon, J.: Das Gitterfasergerüst der Leber unter normalen und pathologischen Verhältnissen, Arch. f. Entwcklngsmechn. Organ. **25**:592-602, 1908.

47. Schmidt, E.: Über die Stutzsubstanz der Leber in normalen und pathologischen Zustände, Beitr. z. path. Anat. **42**:606-615, 1907.

48. Rössle, R., and Yoshida, T.: Das Gitterfasergerüst der Lymphdrüsen unter normalen und pathologischen Verhältnissen, Beitr. z. path. Anat. **45**:110-126, 1909.

49. Russakoff, A.: Über die Gitterfasern der Lunge unter normalen und pathologischen Verhältnissen, Beitr. z. path. Anat. **45**:476-506, 1909.

50. His, 1865, cited by von Ebner [51] and Rühle.[52]

51. von Ebner, V.: Koelliker's Handbuch der Gewebelehre des Menschen, Leipzig, W. Englemann, 1902, Vol. 3, p. 351.

52. Rühle, G.: Über die Membrana propria der Harnkanälchen und ihre Beziehung zu dem interstitiellen Gewebe der Niere, Arch. Anat. u. Entwcklng. Anat. abt., 1897, pp. 153-170.

53. Mall, F.: Das reticulierte Gewebe und seine Beziehungen zu den Bindegewebsfibrillen, Abhandl. math.-phys. Classe königl. Sächs. Gesellsch. Wissensch. **17**:295-338, 1891.

Rössle and Yoshida,[53] Foot and Day,[54] and Rinehart,[55] from 1909 to 1930, supported this view, though Plenck,[56] in his 1927 review, recognized the presence of a hyaline membrane distinct from the abutting reticulum or collagen about renal tubules and sebaceous glands. Other basement membranes he thought of as a cement substance in which more or less numerous argyrophilic reticular fibrils were included.

Foot [57] was perhaps the principal exponent of the view that collagen and reticulum were somewhat different substances. Brief extraction with sodium hydroxide destroyed the argyrophilia of reticulum but left collagen stainable by the acid fuchsin of Van Gieson's mixture, while extraction with boiling water destroyed the fuchsinophilia but left argyrophilia. These findings have been controverted (Nageotte and Guyon [58]; Guyon [59]).

Chemical methods, such as that of Lowry, Gilligan, and Katersky,[60] apparently isolate both collagen and reticulum, as shown by Morrione [61] in 1949.

Periodic Acid-Schiff Method.—It was shown in 1946-1948 by McManus [62] and myself [63] that the periodic acid-Schiff procedure would demonstrate both collagen and reticulum when appropriate techniques were employed. This method depends on the presence of vic-glycols or vic-amino alcohols. These are oxidized by periodic acid to dialdehydes, which may then be demonstrated by a variety of reagents. The most convenient of these is the Schiff sulfite leucofuchsin. Since arylamines also bind firmly with aldehydes, certain amino dyes such as fuchsin, acid fuchsin, and Bismarck brown may be used in place of the Schiff reagent, and colored hydrazines may also be employed.

This periodic acid-Schiff procedure indeed did demonstrate hyaline basement membrane with greater intensity than reticulum or collagen (Gersch and Catchpole,[64] 1949), but when uncounterstained the color was about the same.

54. Foot, N. C., and Day, H. A.: The Occurrence of Reticulum in Tumors, Am. J. Path. **1**:431-443, 1925.

55. Rinehart, J. F.: Reticulum: Its Origin; The Occurrence of Reticulum Fibrils in Capillary Endothelium; A New Method of Demonstration. II. The Finer Capillary Bed, Am. J. Path. **6**:525-540, 1930.

56. Plenck, H.: Über argyrophile Fasern (Gitterfasern) und ihre Bildungszellen, Ztschr. d. gerichtl. Anat. **27**(Pt. 3):302-412, 1927.

57. Foot, N. C.: Chemical Contrasts Between Collagenous and Reticular Connective Tissue, Am. J. Path. **4**:525, 1928.

58. Nageotte, J., and Guyon, L.: Reticulin, Am. J. Path. **6**:631-653, 1930.

59. Guyon, L.: Practical Suggestions for Silver Impregnation of Connective Tissue, Am. J. Path. **7**:47-56, 1931.

60. Lowry, O. H.; Gilligan, D. R., and Katersky, E. M.: The Determination of Collagen and Elastin in Tissues, with Results Obtained in Various Normal Tissues from Different Species, J. Biol. Chem. **139**:795-804, 1941.

61. Morrione, T. G.: Factors Influencing Collagen Content in Experimental Cirrhosis, Am. J. Path. **25**:273, 1949.

62. McManus, J. F. A.: The Histological Demonstration of Mucin After Periodic Acid, Nature **158**:202, 1946; Histological and Histochemical Uses of Periodic Acid, Stain Technol. **23**:99-108, 1948; The Periodic Acid Routine Applied to the Kidney, Am. J. Path. **24**:643-653, 1948; Structure of the Glomerulus of the Human Kidney, ibid. **24**:1259-1269, 1948.

63. Lillie, R. D.: Reticulum Staining with Schiff Reagent After Oxidation by Acidified Sodium Periodate, J. Lab. & Clin. Med. **32**:910-912, 1947; Studies on the Preservation and Histologic Demonstration of Glycogen, Bull. Internat. A. M. Mus. **27**:23-61, 1947; Footnote 41.

64. Gersch, I., and Catchpole, H. R.: The Organization of Ground Substance and Basement Membrane and Its Significance in Tissue Injury, Disease and Growth, Am. J. Anat. **85**:457-522, 1949.

The fact that counterstains made with blue basic anilin dyes changed the red purple of mucin toward the violet and that acid-dye counterstains, such as picric acid, changed the color of collagen to orange led to the use of picromethyl blue, a specific stain for reticulum and collagen, as a counterstain after the periodic acid-Schiff procedure. This gave the allochrome stain,[65] so called because it altered the color of collagen and reticulum from red to bright blue, leaving basement membranes, sarcolemma, Descemet's membrane, and the ocular lens capsule and other periodic acid-Schiff-positive materials in purple to red.

This method has enabled its users to discriminate much more clearly between hyaline basement membranes and the even closely applied (blue) reticular fibrils. It appears from this method, for example, that the red-stained ring fibers about the splenic sinuses are composed of basement membrane material, while the pulp fibrils (blue) are probably true reticulum, and that the liver contains little or no basement membrane, its stroma being almost purely reticular. Pulmonary capillary loops (red) contrast sharply with the blue reticular fibrils. In renal epithelium, fibrin, and hyaline droplets appear in orange, amyloid in purples.

Tryptic digestion [66] appeared to destroy collagen and reticulum more rapidly than the basement membranes when the allochrome method was used as a test procedure after the digestion.

On acid peptic digestion, however, the red-staining basement membrane material proved less resistant than the cyanophil collagen and reticulum, except for the thicker and denser capsule and Descemet's membrane.

A combination of the amine silver methods for reticulum and the periodic acid-Schiff procedure has yielded interesting results. Preparations made by the Wilder, Lillie, or other silver procedures, which showed on close examination quite complete and successful impregnation of renal stroma—the glomerular stroma usually does not impregnate by these methods—were demounted and put through the periodic acid-Schiff procedure. On remounting the basement membranes were found as deep-red hyaline membranes between the black reticulum and the gray-yellow (iron-hematoxylin-picric acid counterstain) epithelium. Spleen presents red sinus ring fibers and black pulp reticulum.

The sarcolemma of striated muscle reacts positively in the periodic acid-Schiff procedure, red in the allochrome method, and on tryptic digestion it swells, its staining reactions weaken, and silver-positive allochroic (blue) fibrils are revealed embedded in its interstitial surface.

The use of electron microscopy in the identification or distinction of collagenous and reticular fibrils affords an interesting approach which properly lies outside of my assigned topic. However, since it has been stated that this approach has conclusively demonstrated the identity of the two types of fibrils, it seems appropriate to offer these comments. Gross's [67] electron-microscope studies of fibrils of rat skin apparently predicated a "reticular" nature of the argyrophil fibrils of the skin.

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65. Lillie, R. D.: The Allochrome Procedure: A Differential Method Segregating the Connective Tissues, Collagen, Reticulum and Basement Membranes, into Two Groups, Am. J. Clin. Path. **21**:484-488, 1951.

66. Lillie, R. D.: Histochemistry of Connective Tissues, Lab. Invest. **1**:30-45, 1952.

67. Gross, J.: A Study of the Aging of Collagenous Connective Tissue with the Electron Microscope, Am. J. Path. **26**:708, 1950.

Gale [68] examined collagen from cirrhotic liver, but the electron microscopy of hepatic reticulum was not specifically recorded. The work of Vanamee and Porter [69] was done on tail tendon and likewise includes no reticulum in Mall's sense.

D. M. Angevine in last year's Macy conference [70] referred to Gross's demonstration that "reticulum" was simply smaller bundles of "collagen," as studied under the electron microscope. I gather from what I have heard of the electron microscopy of the connective tissue fibril that the protagonists of this concept are considering that reticular fibrils and collagenous fibrils are both components of the subcutaneous tissues and that argyrophilia has been considered an important criterion of distinction between these fiber types. From this view I must dissent.

ELASTIC FIBRILS

The presence of yellowish, straight-branching fibrils which did not swell in dilute acetic acid was recognized early in the study of histology.

Normally they stain with acid dyes but are rendered basophilic by treatment with chromic acid or chromates. They quickly reduce osmic acid. In rodents, but not in man, they are often directly Schiff-positive, and hence appear in red in Feulgen nucleal and plasmal preparations, periodic acid-Schiff preparations, and performic- and peracetic-acid-Schiff-reacted material. This direct Schiff reaction is paralleled by a positive Seligman-Ashbel carbonyl reaction (Herman and Dempsey [71]), and the Schiff reactions may all be blocked by prior treatment with phenylhydrazine. Hence, the presence of aldehydic or at least carbonyl groups in rodent elastin would seem reasonably assured. Since the elastin of man stains in common with rodent elastin by most, if not all, of the usually employed empirical methods for elastin, it would seem that these elastins must be basically similar chemically and that the absence of a direct aldehyde reaction in human elastin may depend on some aldehyde conjugation product in man which is not open to react directly with aldehyde reagents. This, of course, is pure speculation but it does indicate a possible line of attack in the elucidation of the iron-resorcin-fuchsin method of Weigert, and the iron-fuchsin method of Gallego. Otherwise, the mechanism of these two methods is quite unknown, while their specificity appears to be quite high.

The other widely used specific method, employing the weak acid orcein in acid alcoholic solution, is also purely empirical. Similarly, the new aldehyde-fuchsin method of Gomori [72] is purely empirical. Gomori uses a new dyestuff formed by the conjugation of paraldehyde and basic fuchsin in acid solution. Besides elastic fibers, the beta cell granules of pancreatic islets and of the anterior hypophysis, mast cell granules, and gastric chief cells stain orthochromatically violet to purple.

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68. Gale: Electron Microscopic Studies of Collagen from Normal and Diseased Tissues, Am. J. Path. **26**:707, 1950.

69. Vanamee, P., and Porter, K. R.: Observations with the Electron Microscope on the Solvation and Reconstitution of Collagen, J. Exper. Med. **94**:255-266, 1951.

70. Transactions of the 1951 Conference on Connective Tissue, Josiah Macy Jr., Foundation, Pakanack Lake, N. J., 1951.

71. Herman, E., and Dempsey, E. W.: The Demonstration of Compounds Containing Carbonyl Groups in Tissue Sections, Stain Technol. **26**:185-191, 1951.

72. Gomori, G.: Aldehyde Fuchsin, a New Stain for Elastic Tissue, Am. J. Clin. Path. **20**:665-666, 1950.

GROUND SUBSTANCE

This is conceived of as an all-pervading matrix in which the fibers are embedded and from which they are thought by some to arise.

With the usual connective tissue stains, usually no ground substance is demonstrated in normal tissue.

In embryonic mesenchyme, in granulation tissue, in umbilical cord, in the stroma of the cock's comb, and in the stroma of certain tumors, such as salivary gland mixed tumor, myxoma, and myxochondroma, stainable amorphous material is demonstrated between the fibrils.

This material has been referred to as connective tissue mucin. Chemically it contains hyaluronic acid, some chondroitin-sulfuric acid, and possibly other sulfuric esters.

Mallory [11] recommended mucihematin for the demonstration of ground substances as well as the thiazin dyes which exhibit metachromasia. Holmgren and Wilander [73] and others have insisted that lead acetate be used in fixing material for the demonstration of connective tissue mucins. This insistence would put the use of methods for the study of these substances practically outside the domain of the practice of diagnostic pathology, unless it be with the aid of prophecy or clairvoyance.

Fortunately, quite good results can be obtained with such dyes as thionin, safranin O, toluidin blue, Bismarck brown, after fixation with alcoholic formalin, which is fairly commonly used, and even with more or less acid aqueous formalin.

I have had brilliant results with safranin after acetic alcohol formalin, using a sequence Weigert-acid-iron chloride-hematoxylin-fast green FCF-safranin O procedure.[41]

Angevine [70] refers to the metachromasia of connective tissue ground substance as due to the presence of mucopolysaccharides such as mucoitin- and chondroitin-sulfuric acids and hyaluronic acid. The recent demonstration [74] of a thermostable chondromucinase in testicular hyaluronidase has enabled us to decide that the metachromatically staining connective tissue matrix is probably a chondromucin. I do not deny that hyaluronic acid is present in this matrix, but it now appears that we have no proved method of demonstrating it histochemically. Both the metachromasia and the iron-absorption reactions are apparently reduced as greatly by the chondromucinase alone as by the mixed enzymes containing hyaluronidase as well.

The dialyzed-iron method of Hale [75] and the mannitol-ferric chloride procedures of Lillie and Mowry [76] are apparently simply manifestations of the ability of epithelial and connective tissue mucins, as well as collagen and reticulum, to fix iron. It is noteworthy that treatment of sections with ferric salts before application of

73. Holmgren, H., and Wilander, O.: Beitrag zur Kenntnis der Chemie und Funktion der Ehrlichschen Mastzellen, Ztschr. mikr. anat. Forsch. **42**:242-278, 1937.

74. Lillie, R. D.; Emmart, E. W., and Laskey, A. M.: Chondromucinase from Bovine Testis and the Chondromucin of the Umbilical Cord, A. M. A. Arch. Path. **52**:363-368, 1951.

75. Hale, C. W.: Histochemical Demonstration of Acid Polysaccharides in Animal Tissue, Nature **157**:802, 1946.

76. Lillie, R. D., and Mowry, R. W.: Histochemical Studies on the Absorption of Iron by Tissue Sections, Bull. Internat. A. M. Mus. **30**:91-94, 1949.

the Gomori-Burtner [77] argentaffin cell procedure with methenamine silver converts the procedure into a quite selective connective tissue fiber and epithelial mucin stain. I have not used this procedure specifically in the study of connective tissue or its mucins. The observations are incidental to an inquiry into the chemical properties of the enterochromaffin substance.

As I reach this point in my presentation I find I have neglected the polychromatic phosphotungstic, phosphomolybdic, and molybdic acid-hematoxylin procedures.[78] These are primarily stains for intracellular structures and fibrils. The phosphotungstic acid may be useful in contrasting fibrin (pink) and collagen (blue).

Their mode of operation is obscure. Their results depend to a considerable extent on the fixation method employed, and without the special fixing procedures recommended, though Peers' mercuric chloride ($HgCl_2$) mordant helps with the phosphotungstic acid, their success is often problematical.

FIBRINOID

I must confess that I have no clear understanding of the nature of the substance or substances referred to under this name.

I had used the term "fibrinoid" to designate a staining reaction of collagen, renal hyaline droplets, and renal casts to the Weigert fibrin variant of Gram's method. That apparently is not what has been meant by the term "fibrinoid" in the study of lupus erythematosus, rheumatoid nodules, Aschoff bodies, and the aging placenta.

Bennett, in the first Macy conference on connective tissue [70] traced the term fibrinoid degeneration or alteration back to Neumann (1880). "Fibrinoid" has been defined as an oxyphilic, acellular, homogeneous, refractile substance. According to Altshuler and Angevine,[79] it is also metachromatic with toluidin blue, and stains intensely with methods for polysaccharides. It is seen in various places—for example, in the pulmonary alveoli in some forms of prolonged pneumonitis. Phosphotungstic acid-hematoxylin tends to color it blue in part, orange to yellow in other fibrillar parts. An anilin blue stain, presumably of the Mallory type, shows red material between blue (collagen) fibrils. Fibrin is also colored red by this type of stain. Dempsey [70] in discussion referred to cyanophilia by "azan" and a periodic acid-Schiff reaction and to the identity of these reactions and those of true fibrin. Dempsey, however, stressed the orthochromasia of fibrin.

Angevine and Dempsey were apparently regarding the periodic acid-Schiff result as a mucopolysaccharide reaction.

Sokoloff, Mund, and Kantor [80] determined that the isoelectric points of fibrin, collagen, and "fibrinoid" from rheumatoid nodules, Aschoff bodies, and term placenta, were closely similar when determined by staining in fast green FCF at vary-

77. Burtner, H. J., and Lillie, R. D.: A 5 Hour Variant of Gomori's Methenamine Silver Method for Argentaffin Cells, Stain Technol. **24**:225-227, 1949.

78. Mallory.[11] Lillie.[41]

79. Altshuler, C. H., and Angevine, D. M.: Histochemical Studies on the Pathogenesis of Fibrinoid, Am. J. Path. **25**:1061-1077, 1949. Footnote 70.

80. Sokoloff, L.; Mund, A., and Kantor, T. G.: The Affinity of Fibrinoid for Acid Dyes, Am. J. Path. **27**:1037-1045.

ing controlled pH levels. Singer and Wislocki,[81] using orange G and methylene blue as representative acid and basic dyes at controlled pH levels, recorded essentially identical uptake of these dyes by fibrin and fibrinoid over the pH range 1.5 to 6.8.

Altshuler and Angevine [79] recorded for fibrinoid from various lesions a variable metachromasia to orthochromasia to toluidin blue, purple staining with crystal violet, yellow to orange with phosphotungstic acid-hematoxylin, yellow, orange, or red with Van Gieson, red with the periodic acid-Schiff method, and yellow orange with Sakaguchi's arginine method. They stated that the fibrin (of cardiac vegetations) usually failed to react with the periodic acid-Schiff procedure, though in areas adjacent to fibrinoid it reacted. In earlier phases the metachromasia was destroyed by hyaluronidase (in Aschoff nodules, synoviae, and lupus erythematosis).

Their more recent paper [82] apparently used identical methods and attached the same significance to them.

MYXEDEMA

Brewer [83] in 1951 reported on the histologic and histochemical aspects of a case of essentially untreated human myxedema. The interstitial exudate in the tongue reacted positively in the periodic acid-Schiff procedure and was in part strongly metachromatic to toluidin blue and still basophilic at pH 2.5. Bull testis hyaluronidase reduced the basophilia of the tongue deposits only slightly in their periphery, and not at all centrally. Malt diastase digestion, which abolished the metachromasia of cartilage but not its periodic acid-Schiff reaction, did not affect the metachromasia of the myxedema exudate. The exudate in heart muscle differed in not taking up methylene blue below pH 5, in its lack of metachromasia, and in its much weaker staining with hematoxylin and lack of staining with mucicarmine. Dermal exudate, so far as tested, resembled that in the tongue.

81. Singer, M., and Wislocki, G. B.: The Affinity of Syncytium Fibrin and Fibrinoid of the Human Placenta for Acid and Basic Dyes Under Controlled Conditions of Staining, Anat. Rec. **102**:175-193, 1948.

82. Altshuler, C. H., and Angevine, D. M.: Acid Mucopolysaccharides in Degenerative Disease of Connective Tissue, with Special Reference to Serous Inflammation, Am. J. Path. **27**:147-156, 1951.

83. Brewer, D. B.: Myxoedema: An Autopsy Report with Histochemical Observations on the Nature of the Mucoid Infiltrations, J. Path. & Bact. **63**:503-512, 1951.

Books

HISTOLOGIE UND MIKROSKOPISCHE ANATOMIE DES MENSCHEN. By Prof. Dr. W. Bargmann, a. o. Professor der Anatomie an der Universität Kiel. Pp. 563, with 412 illustrations. Price 45 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a), Stuttgart O; Agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

This is an adequate textbook of organology, based largely on a body of knowledge contributed or compiled by German histologists. Perhaps it is a sign of the times that references to important contributions made to modern histology from other countries are scarce and inadequate. One might, perhaps, consider it commendable that a technically well-executed textbook appears under a German imprint during difficult circumstances of reconstruction. But from the level of international scholarship and competition, this work will not necessarily have to be reckoned with.

This second volume considers organs and organ systems, the fundamental tissues having been presented in the first volume. There is good evidence for a determined interest and effort to correlate chemical, physiological, and pathological concepts with microscopic structure. But many subchapters are too succinct and incomplete. This is particularly evident in the descriptions of microscopic features of the skeleton and of the various categories of joints. The author appears unaware of important newer contributions and concepts, for example, concerning the regeneration of lymph nodes, the structure and function of the spleen (Knisely), and cutaneous pigmentation. Among the best chapters are those describing the histologic aspects of endocrine glands and of the thymus, in which field the author has contributed to Möllendorff's Handbook of Microscopic Anatomy. The arteriovenous anastomoses are considered with more than usual detail. Brief indications of age changes in arteries (calcium deposits beginning in childhood) might be of interest to pathologists. The book contains many numerical data on the relative vascularizations of gray and white brain matter, of skeletal muscle and of the cardiac walls, on tensile strength of tendons, or on amounts of secretions produced by various glands. Fat and protein resorption are well considered. The histology of the sense organs and of the central nervous system is included. The quality of the figures is good. Many are reproductions of drawings rather than photomicrographs. The only illustrations in color concern blood cells. The book has a good index.

REACTION TO INJURY: II. THE REACTIONS OF SUBMISSION AND ADAPTATION AND THE DISEASE ENTITIES ARISING OUT OF THEIR ELABORATION. By Wiley D. Forbus, M.D., professor of pathology, Duke University; pathologist to Duke Hospital. Pp. 1110, with 836 illustrations, 54 of which are in color. Price $20. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1952.

ADVANCES IN MEDICINE AND SURGERY. From the Graduate School of Medicine of the University of Pennsylvania. Pp. 441, with 43 illustrations. Price $8. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

PATHOLOGY OF THE CELL. By Gordon Roy Cameron, M.B., D.Sc. (Melb.), F.R.C.P., F.R.S., director of the Graham Research Laboratories and professor of morbid anatomy at University College Hospital Medical School in the University of London. Pp. 840, with 41 illustrations and 64 plates. Price $22.50. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1952.

SURGICAL FORUM: PROCEEDINGS OF THE FORUM SESSIONS, THIRTY-SEVENTH CLINICAL CONGRESS OF THE AMERICAN COLLEGE OF SURGEONS, SAN FRANCISCO, NOVEMBER, 1951. Pp. 667, with 290 figures. Price $10. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

TRANSACTIONS OF THE AMERICAN GOITER ASSOCIATION: 1951 ANNUAL SESSION, MAY 24, 25, AND 26. Pp. 506, with numerous illustrations and tables. Price $12. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1952.

BIOCHEMISTRY FOR MEDICAL STUDENTS. By William Veale Thorpe, M.A. (Cantab.) Ph.D. (Lond.), reader in chemical physiology, University of Birmingham. Fifth edition. Pp. 528, with 41 illustrations. Price $6. J. B. Lippincott Company, 227-231 S. Sixth St., Philadelphia 5; 2083 Guy St., Montreal, Aldine House, 10-13 Bedford St., London, W.C. 2, 1952.

ANGEWANDTE UND TOPOGRAPHISCHE ANATOMIE. By Gian Töndury, ordentlicher Professor der Anatomie an der Universität Zürich. Pp. 416, with 369 illustrations. Price 70 German marks ($16.65). Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; Agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

The outstanding features of this beautiful volume by the director of the Anatomical Institute of the University of Zürich reside in the accurate, scholarly text and in the excellent illustrations. The author's German is clear, highly readable, and should present little difficulty to anyone interested in good anatomical descriptions. There is no dreariness or overemphasis of anatomical minutiae. The textual organization and clarity of presentation make even difficult relationships appear simple and understandable. The embryological approach to many basic situations is also helpful. Diligent search by this reviewer has failed to uncover serious mistakes or omissions in factual information. The over-all accent of the volume is on topography rather than on surgically applied anatomy, although there are many practical indications for surgical procedures and for the spread of pathological processes.

The book was printed in Switzerland, and the high technical standards are reflected especially in the unsurpassed quality of some 95 multicolored plates. These constitute truly an atlas in themselves. The original figures were made by a competent medical illustrator with thorough anatomical training and clinical understanding. Particularly beautiful are the full-page illustrations of various mediastinal aspects, of the abdominal and pelvic autonomic nerve plexuses, of cervical, facial and intracranial situations, and of hand and foot. Numerous x-ray reproductions are dispersed throughout the book. The remainder of the figures are good semidiagrammatic line drawings.

The reviewer feels compelled, however, to warn American readers with regard to the nomenclature employed. The B. N. A. terminology, which has been largely retained by American anatomists and in which the younger pathologists and surgeons have been trained, was abandoned by the author. He used, instead the Jena Nomenclature with certain compromises. This is a decision with inherent dangers and disappointments to American and English readers. The fault lies perhaps less with the author than with the present nonexistence of an international agreement on anatomical nomenclature.

The discrepancies may appear greater than they are in fact, as clear-cut Latin descriptive terms are the basis of all recent modifications.

The scholar in anatomy, pathology, and surgery will want to own or to consult this book. It has a good index. The bibliography is restricted and refers mainly to standard works of international reputation in this field.

SURGERY AND THE ENDOCRINE SYSTEM: PHYSIOLOGIC RESPONSE TO SURGICAL TRAUMA —OPERATIVE MANAGEMENT OF ENDOCRINE DYSFUNCTION. By James D. Hardy, M.D., assistant professor of surgery at the University of Tennessee Medical College. Pp. 153, with 43 illustrations. Price $5. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C.2, 1952.

CALLANDER'S SURGICAL ANATOMY. By Barry J. Anson, M.A., Ph.D. (Med. Sc.), professor of anatomy at Northwestern University Medical School, and Walter G. Maddock, M.S., M.D., Elcock professor of surgery at Northwestern University Medical School. New third edition. Pp. 1074, with 929 illustrations. Price $14. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

LES TUMEURS ARTICULAIRES MALIGNES. By R. De Grailly, professeur à la Faculté de Bordeaux Médecin des Hôpitaux et de la Fondation Bergonié, and Henry Leger, chef des travaux d'anatomie pathologique à la Faculté Médecin des Hôpitaux. Paper bound. Pp. 118, with 7 illustrations. Price 600 francs. Masson & Cie, 120 Boulevard Saint-Germain, Paris, 6e, 1952.

DER EINFLUSS DES NERVENSYSTEMS AUF BAU UND TÄTIGKEIT DER GESCHLECHTSORGANE DES MENCHEN. By Prof. Dr. Med. et Phil. H. Stieve. Pp. 191, with 93 illustrations. Price 36 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; Agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Avenue, New York 16, 1952.

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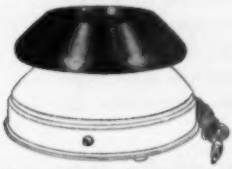

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